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27.—A COMPARISON OF SOME EUROPEAN AND AMERICAN VIRUS DISEASES OF THE POTATO. By PAUL A. MURPHY, Sc.D., and ROBERT MCKAY, A.R.C.Sc.I., Albert Agricultural College, Glasnevin, Dublin.

[*Authors alone are responsible for all opinions expressed in their Communications.*]

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No. 21.

REPORT OF THE IRISH RADIUM COMMITTEE FOR THE YEAR 1931.

[Read MAY 24. Published separately JUNE 6, 1932.]

16,756 MILLICURIES of radon were issued for medical purposes during the year 1931. This is a considerable reduction on the record amount, 21,533 millicuries, issued during 1930, but larger than the quantity for any previous year. The decrease is attributable to the death, early in the year, of Dr. Walter Stevenson, as recorded in the Committee's Report for 1930. His work had entailed the use of very large supplies of radon. The acquisition by the Radium Committee, early in the year, of a further 224 milligrams of Radium has brought the supply of the element up to about 1,130 milligrams. The modified radon apparatus mentioned in last year's Report has proved highly successful in use.

The Committee were obliged to accept with regret the resignation of Dr. K. C. Bailey, F.T.C.D., from the staff of the Institute, owing to the increasing demands made upon his time by his other duties. The vacancy thus created has been filled by the appointment of Mr. G. Stevenson.

Mr. F. R. Cleary and Dr. T. A. Bouchier-Hayes were appointed to the Radium Exhibitions falling vacant during the year.

Reports from some of the largest users of radon supplied by the Institute are printed below.

REPORT BY OLIVER CHANCE, M.B., B.CH.,
Richmond, Sir Patrick Dun's, and Coombe Hospitals.

LIST OF CASES TREATED.

MALIGNANT, 160.

RODENT ULCER	32	CARCINOMA CERVIX UTERI ...	20
CARCINOMA SKIN	18	„ CORPUS UTERI ...	1
„ LIP	13	„ VULVA	2
„ TONGUE	26	„ BREAST	3
„ CHEEK	4	„ RECTUM	4
„ GUM	3	„ ANUS	2
„ PALATE	3	„ THYROID	1
„ FLOOR OF MOUTH ...	9	TERATOMA OF TESTES ...	1
„ FAUCES, TONSIL, AND PHARYNX ...	9	PAROTID TUMOURS	2
„ LARYNX	2	BRAIN TUMOURS	1
		SARCOMA	4

NON-MALIGNANT, 37.

TUBERCULAR PERITONITIS ...	13	MENORRHAGIA	1
„ ADENITIS ...	2	LUPUS	3
EXOPHTHALMIC GOITRE ...	1	KELOID	5
MENOPAUSAL HÆMORRHAGE ...	5	NAEVI	7

Number of cases of all types of malignant disease:—

1.	Total number seen during 1931	329
2.	Number treated during 1931	267
3.	Do. by Surgery alone	56
4.	Do. by Radium alone	106
5.	Do. by X-rays alone	32
6.	Do. by Surgery and Radium ...	27
7.	Do. by Surgery and X-rays ...	19
8.	Do. by Radium and X-rays ...	24
9.	Do. by Surgery and Radium and X-rays	3

(*N.B.*—As some of the cases treated with radium or X-rays were referred by hospitals from which figures for the surgical treatment of cancer were not obtained, the number of cases treated by surgery alone (56) is misleading. Double this figure, *i.e.*, 112, would be approximately correct.)

MALIGNANT CASES.

Rodent Ulcers, 36.

Total number of cases, 36. Treated with radium, 32. Treated by other means, 2. Unsuitable, 2.

In the treatment of rodent ulcers implantation is, as a rule, preferred to surface application, and a long time period is usually employed.

The cases have been collected into two groups: (*a*) those in which no previous treatment had been given, and (*b*) those in which this had failed or had been followed by recurrence of the disease. As one would expect, cases respond very much better to radium if no previous treatment has been given.

TABLE 1.—*Cases which had received no previous treatment.*

Method of treatment used.	No. of cases treated.	Well.	Improved.	Unimproved.	Untraced.
Radium alone ...	24	21	1	—	2
Radium and Surgery ...	1	1	—	—	—
X-ray alone ...	2	2	—	—	—

The 2 cases treated by X-rays were peculiar. They both were of long duration (18 years and 24 years); and they both occurred in the skin of the

lumbar region. In both cases the ulcers were of very large size, but superficial. On account of their size (one was as big as a dinner plate), X-rays were preferred to radium.

TABLE 2.—*Cases which had previous treatment.*

Nature of previous treatment.	No. of cases.	Well.	Improved.	Unimproved.	Untraced.
Radium ...	1	—	—	1	—
Surgery ...	2	—	—	1	1
X-rays ...	4	2	—	2	—

Carcinoma of Skin, 19.

Total number of cases seen, 19. Treated, 18. Unsuitable, 1.

No. of cases.	Well.	Primary growth well, but glands involved.	Unimproved.	Untraced.
18	12	2	2	2

Both the unimproved cases had been previously treated by local applications ("cancer plasters").

Buccal Carcinoma, 93.

Total number of cases seen, 93. Treated, 71. Unsuitable, 22.

The primary growths were treated by implantation, except in a few cases (carcinoma of the gums and hard palate), in which radium bearing plates were used. The element or radon was screened by 0·6 mm. of platinum, and the needles had an intensity of 0·66 mgrs. or 1·8 mcs. per cm. Birkett's technique of implantation and dosage was employed.

Surgery was preferred to radium in the treatment of four cases of lingual cancer. In all a small growth had occurred on a tongue, which was the site of severe chronic glossitis. It was excised by diathermy, because one felt that the condition of the tongue rendered irradiation inadvisable. Two of these cases are still free from disease.

All cases were asked to report 8 weeks after the primary growth had been treated. If any induration was present in the treated area, this was excised by diathermy. If mobile glands were present, they were removed by a block dissection. Inoperable glands were treated by implantation, or, if the primary growth was clinically a sensitive one, by high voltage X-rays. In some cases the latter has been of considerable palliative value, and appeared to be quite as efficient as surface radium treatment. The use of radium bearing moulds has been discontinued. For economic reasons, it is not possible to work at a greater skin distance than 3 cms., and it is felt that under these conditions a sufficiently large percentage depth dose is not obtained.

The intra oral cases have been grouped according to the anatomical region in which the growth originated. This is only a rough classification, as most of the cases when first seen involved more than one region, and in many it was impossible to be sure of the point of origin of the tumour. No case has been included in more than one group.

One case of cancer of the upper lip deserves special mention, because of the number of important questions it raises.

An ulcer only 7 mm. in diameter was present on the upper lip for 3 weeks. It was so small that the diagnosis was doubtful. After surface treatment it disappeared, leaving no mark of any sort. The patient did not attend regularly for inspection. When seen six months later, the glands on one side of the neck were involved, and were removed by block dissection. A few months later there were enlarged glands on both sides of the neck, in both supra-clavicular region and in one axilla. The patient has since died.

Should a biopsy have been done?

If so, would the treatment have been different?

If a unilateral block dissection had been done while there were still no palpable glands, would the patient's life have been saved?

Carcinoma of Lip, 14.

Total number of cases, 14. Treated, 13. Unsuitable, 1.

	No. of cases.	Well.	Primary well, but glands involved.	Unimpr.	Untraced.	Dead.
(*a*) No gland involvement	8	6	—	—	1	1
(*b*) Glands enlarged but mobile ...	3	1	1	—	—	1
(*c*) Glands enlarged and inoperable ...	2	—	1	1	—	—

Carcinoma Tongue, 38.

Total cases seen, 38. Treated, 30—by radium or radium and surgery, 26; by surgery alone, 4; unsuitable, 8.

	No. of cases.	Well.	Primary well, but glands involved.	Unimpr.	Untraced.	Dead.
(*a*) No enlarged glands	11	6	1	1	1	2
(*b*) Glands enlarged but mobile ...	9	2	1	2	2	2
(*c*) Glands enlarged and inoperable ...	6	0	1	—	2	3

Carcinoma Cheek, 5.

Cases seen, 5. Treated, 4. Unsuitable, 1.

	No. of cases.	Well.	Primary well, but glands involved.	Unimpr.	Untraced.	Dead.
(*a*) No enlarged glands present ...	3	1	—	1	—	1
(*b*) Enlarged mobile glands	1	—	—	—	—	1
(*c*) Enlarged inoperable glands ...	—	—	—	—	—	—

Carcinoma Gum, 5.

Cases seen, 5. Treated, 3. Unsuitable, 2.

	No. of cases.	Well.	Primary well, but glands involved.	Unimpr.	Untraced.	Dead.
(*a*) No enlarged glands present ...	2	1	—	—	—	1
(*b*) Enlarged glands but mobile ...	1	—	—	1	—	—
(*c*) Enlarged inoperable glands ...	—	—	—	—	—	—

Carcinoma Palate, 3.

Cases seen, 3. Treated, 3. Unsuitable, 0.

	No. of cases.	Well.	Primary well, but glands involved.	Unimpr.	Untraced.	Dead.
(*a*) No enlarged glands present ...	3	3	—	—	—	—
(*b*) Enlarged mobile glands	—	—	—	—	—	—
(*c*) Enlarged inoperable glands ...	—	—	—	—	—	—

All three cases very early, and highly radio-sensitive. As in the case of cancer of the gum, radium bearing moulds were used. Two of the three cases occurred in women under 30.

Carcinoma Floor of Mouth, 14.

Treated, 9. Unsuitable, 5.

	No. of cases.	Well.	Primary well, but glands involved.	Unimpr.	Untraced.	Dead.
(*a*) No enlarged glands ...	5	2	—	1	1	1
(*b*) Glands enlarged and mobile ...	2	—	—	1	—	1
(*c*) Glands enlarged and inoperable ...	2	—	—	1	—	1

Carcinoma Fauces, Tonsil and Pharynx, 14.

Treated, 9. Unsuitable, 5.

	No. of cases.	Well.	Primary well, but glands involved.	Unimpr.	Untraced.	Dead.
(*a*) No enlarged glands ...	3	1	—	—	1	1
(*b*) Glands enlarged and mobile ...	4	1	1	—	—	2
(*c*) Glands enlarged and inoperable ...	2	—	—	1	1	—

One case of Shimke's tumour of the tonsil was seen. The primary growth extended across the mid-line, and a secondary swelling, size of a tangerine orange, was present in the neck. Both disappeared as the result of a small dose of X-rays.

Carcinoma of Larynx, 2.

Two intrinsic growths of the larynx were treated with radium by fenestration of the thyroid cartilage (Harmer's technique). One patient is free from disease, and his voice is scarcely distinguishable from normal. The second died very shortly after operation.

Carcinoma Cervix Uteri, 23.

The implantation technique described last year is still used, the only alteration being that two applications, lasting four days, with seven to ten days' interval between them, have been substituted for a single application lasting eight days. Whenever possible, this is followed up eight to ten weeks later by deep X-ray therapy to the entire pelvis.

Two cases of suspected early malignancy were referred for radium treatment. After the first application sections of the growth were taken, and the pathological reports were negative. Nevertheless, in one of these cases the treatment was continued, because it was clinically a typical cancer. It has not been included in the statistics.

TABLE OF CASES.—23.

Operable, 6.

Name.	Duration of Symptoms.	Section.	Condition in February, 1932.
Mrs. D., 34 years ...	4 months	—	Well.
*Mrs. C., 60 years ...	2 „	—	Well.
Mrs. G., 53 years ...	2½ „	—	Well.
Mrs. O'R., 47 years ...	5 „	—	Dead.
Mrs. M., 34 years ...	6 „	—	Advanced pelvic involvement.
Mrs. M., 62 years ...	3 „	+	Well.

Borderline, 6.

Name.	Duration of Symptoms.	Section.	Condition in February, 1932.
Mrs. M'N.	18 months.	—	Well.
Mrs. K.	4 „	+	Well.
*Mrs. F.	8 „	+	Untraced.
Mrs. T.	4 „	—	No symptoms, but pelvic involvement.
Mrs. R., 84 years ...	12 „	—	Well.
Mrs. M., 30 years ...	4 „	—	Dead.

Inoperable, 8.

Mrs. C., 46 years ...	8 „	—	Dead.
Mrs. B., 62 years ...	6 „	—	No symptoms, but pelvic involvement present.
Mrs. K., 52 years ...	5 „	—	Well.
Mrs. R., 46 years ...	3 „	—	Advanced general carcinoatosis.
Miss O'D., 35 years ...	4 „	+	Well.
*Mrs. N., 49 years ...	3 „	+	Untraced.
Mrs. M'G., 40 years ...	4 „	—	Untraced.
Mrs. M., 48 years ...	6 „	—	No symptoms, not examined.

Mortality nil.

Hopeless, 4.

Mrs. R., 55 years ...	10 „	—	Dead.
Mrs. W., 41 years ...	9 „	—	Untraced.
Mrs. S., 45 years ...	2½ „	+	Untraced.
Mrs. P., 60 years ...	14 „	+	Dead.

* Refused to complete treatment.

The operability rate was decided by referring gynæcologist, who also made the biopsy in those cases where this was done.

Carcinoma of Corpus Uteri, 1.

One case of recurrence in the anterior vaginal wall, four years after operation, was treated by implantation. This case has not been traced.

Carcinoma of Vulva, 3.

Treated, 2. Unsuitable, 1. Well, 0. Untraced, 1.

Of the two cases treated, one is untraced and the other is still active; both suffered considerably from post-operative vaginitis and severe inflammation of the vulva. I believe that excision is the treatment of choice in these cases.

Carcinoma of Breast, 17.

During the past year the Stockholm technique has been largely adopted. As this is a combination of deep X-ray therapy and operation, radium has

scarcely been used for these cases. It is still preferred to X-rays in the treatment of multiple skin metastases. Needles are also implanted into the axillary glands in some inoperable cases. Three cases of multiple skin metastases were treated by a surface application of radium; in all the local condition has temporarily cleared up.

Carcinoma Rectum, 10.

The results here were again unsatisfactory. Four cases were treated by implantation after removal of the coccyx; in all the disease is still active, and only one case shows any improvement.

Three cases were treated by deep X-ray therapy. There was no evidence of improvement in any case.

Carcinoma Arms, 2.

Two advanced cases were treated by colostomy, followed by radium implantation. Both are apparently free from disease.

Teratoma of Testis, 1.

One case was treated by excision, followed by surface application to the inguinal region. There is no evidence of disease twelve months after treatment.

Carcinoma of Thyroid, 1.

One advanced case was treated by implantation, followed by surface application. Although there was a definite improvement locally, the patient died four months later.

Brain Tumours, 1.

A case of a large hæmangioma of the meninges in a child was treated by surface application at a skin distance of 3 cms.

This patient is well, but as a decompression had been done before radium was applied, there is no evidence that the latter was effective.

Tumours of Parotid, 2.

Both were treated by removal of the tumour, combined with implantation of radium. One, a mixed cell tumour, has remained free from disease; the other, a squamous celled cancer has recurred, and is at present receiving further treatment.

Sarcoma, 14.

Cases seen, 14. Treated, 11—radium alone, 1; radium and X-ray, 3; X-ray alone, 7.

At present three of these cases appear to be free from disease.

1. Fibra-sarcoma of ulna, amputation refused. The growth was excised, and needles implanted in the tumour bed. One course of deep X-rays has been given since then.

2. Melanotic sarcoma (skin of back). Treated by intensive X-ray therapy. No evidence of growth five months later.

3. Mixo-sarcoma of sole of foot. Treated by implantation. No evidence of growth twelve months afterwards. Patient has, however, a severe radium burn, which is healing very slowly.

Non-Malignant Cases.

Tubercular Peritonitis, 13.

In the treatment of these cases the abdominal wall is divided into 4–6 areas. A mould 2 cms. thick, bearing about 100 millicuries, split into 6–10 foci, screened by 3·0 mm. brass, is applied over each area in turn. The time periods are successively increased, so that the skin covering the abdomen shows mild general erythema. The whole application takes from 7–14 days.

It is difficult to see how this technique can have any direct effect on the peritoneum and abdominal contents. Nevertheless, in practically every case treated there was definite evidence of improvement within 48 hours of the commencement of treatment. Pyrexia was lessened, pain was eased, and the patient usually felt better. If abdominal distension was present, it was usually less within a week.

The patient's general condition, weight, and appetite are carefully watched, and, if all these are improving, no further treatment is given. Occasionally a single application is sufficient, but usually 3–4 treatments are required.

One has gained the definite impression that radium gives infinitely better results than X-ray therapy in the treatment of these cases.

Table of Cases.

Total treated.	Well.	Still under treatment.	Untraced.	Dead.
13	6	2	1	4

Tubercular Adenitis, 2.

As a rule, X-ray is preferred to radium in the treatment of this condition, but the latter was used in two cases, both of which responded readily, and are now free from disease.

Exophthalmic goitre, 1.

This case, a young girl of eighteen, had completely failed to respond to medical treatment. She was much too ill to be operated upon or to be moved from the ward to the X-ray department. Her pulse was about 180, weak and irregular, and she was tossing and gasping like a fish out of water.

It was decided that her only hope lay in surface treatment with radium. The patient died, however, within three days of the commencement of treatment.

Menopausal Hæmorrhage, 5.

An intra uterine dose of 1,600 mgr. hrs. was given, and in all cases bleeding has ceased.

Menorrhagia, 1.

This was a severe case. After the usual methods of treatment had been tried, the patient's condition became so bad that both ovaries were removed. Even this did not cause the cessation of hæmorrhage. An intra uterine application of 1,500 mgr. hrs. was then given.

The patient still remains free from bleeding, 12 months later.

Lupus, 3.

Radium was preferred to other methods of treatment in three very early cases. A desquamation dose was given.

Two cases remained free from disease, and the condition of the third case has greatly improved.

Keloids, 3.

Treatment was only attempted when the keloid was young and vascular. Rather intensive doses of gamma-rays were used, so that desquamation occurred.

Three cases were treated; all are much improved, but in all some keloid element remains.

Naevi, 7.

Only strawberry naevi were treated by radium. This was screened by 1 mm. Al. and about half an erythema dose was given at monthly or two monthly intervals.

Two of these cases are well. The remaining five are greatly improved, but are still under treatment.

REPORT BY JOHN A. GERAGHTY, M.B.,

Mater Misericordiae Hospital.

MALIGNANT DISEASE.

Intern patients	132
Extern patients	No record.
Treated by Surgery only	Figure not available.
„ „ Radium only	0
„ „ X-ray only	19
„ „ Surgery and Radium	1
„ „ Surgery and X-ray	15
„ „ Surgery, Radium, and X-ray	2
„ „ X-ray and Radium	4

1. 14/1/'31.—Female, aged 50 years. Small malignant epulis right lower jaw. Treated as follows:—*Superior aspect.*—Curved applicator, 2 × 3 cms.;

filtration, 0·3 mm. platinum + 5 mm. rubber; 245 mc. hours. *Anterior cutaneous aspect.*—Flat applicator, 6 × 4 cms.; filtration, 2·0 mm. lead + 5·0 mm. rubber; 880 mc. hours. *Submental.*—Flat applicator, 6 × 4 cms.; filtration, 2·0 mm. lead + 5·0 mm. rubber; 1,460 mc. hours. 11/3/'31.—Primary focus healed.

2. 16/2/'31.—Male, aged 52 years. Inoperable carcinoma of rectum. 16/2/'31–13/3/'31.—Deep X-ray therapy in fractional doses. 26/3/'31–30/3/'31.—Tubular applicator introduced into rectum; filtration, 0·3 mm. platinum + 1·0 mm. lead + 3·0 mm. rubber; 2,558 mc. hours. Has not reported since treatment.
3. 4/6/'31.—Male, aged 13 months. Mixed naevus left cheek. 4/6/'31 to 6/6/'31.—Flat applicator, 0·1 mm. lead filter; 186 mc. minutes, in equal daily doses. 20/7/'31 to 22/7/'31.—257 mc. minutes. 6/10/'31 to 8/10/'31.—322 mc. minutes. 10/12/'31 to 12/12/'31.—510 mc. minutes. 26/2/'32.—Healed.
4. 30/10/'31.—Female, aged 43. Recurrent nodule of carcinoma in vaginal scar. A little thickening felt in left broad ligament. Uterus and vault of vagina removed 7 months ago for carcinoma of uterine cervix. Two radon needles implanted in periphery of nodule. Filtration, 0·3 mm. platinum; 432 mc. hours. 28/10/'31.—Recurrence healed. 9/12/'31 to 22/12/'31.—Deep X-ray therapy in fractional doses to pelvis. 26/2/'32.—Feels very well. Has gained 7 lbs. Recurrence healed. No thickening to be felt in left broad ligament.
5. Male, aged 50 years. Squamous cell carcinoma behind left ear. No palpable glands. 21/12/'31.—Implantation of 9 needles; filtration, 0·3 mm. platinum; 3,285 mc. hours. 19/1/'32 to 26/1/'32.—Deep X-ray therapy to lymphatic areas. 3/2/'32.—Primary focus healed.

Report by Oswald J. Murphy, M.B., B.Ch., B.A.O.,

St. Vincent's Hospital, Dublin.

Cases Treated during the Year 1931.

Malignant, 84.

Rodent Ulcer	27	Carcinoma of Uterus ...	3
Carcinoma of Tongue ...	8	Carcinoma of Palate, Tonsil,	
„ Cervical Glands	10	Pharynx, &c.	8
„ Breast ...	4	„ Skin ...	15
Carcinomatous Secondaries (following Removal of Breast) ...	6	Sarcomata	1
		Brain Tumours	2
		Branchial Carcinoma ...	1

NON-MALIGNANT, 40.

WARTS	10	LUPUS VULGARIS	1
TUBERCULAR GLANDS	6	HODGKIN	3
ANGIOMATA	15	ENLARGED SPLEEN	1
KELOIDS	4		

Rodent Ulcers.

None of the cases were very extensive, and all reacted perfectly to surface applications alone. Four of the excavating type required second treatments, and up to date all appear cured.

Dosage varied from 600–1,000 millicurie-hours, with full-strength applicators at a distance of 1 cm., and screened by 1·5 mm. lead.

Carcinoma of Tongue, 8.

C. M., age 65. — 1 sq. cms. ulceration on middle $\frac{1}{3}$, small palpable cervical glands. 8 platinum needles (·5 mm.), containing 1·8 mc. each, and left embedded for 7·7 days. Two weeks later 7,000 mc. hours were given over glands.

J. O'K., age 80.—2 sq. cms. ulceration on posterior $\frac{1}{3}$, involving alveolus. Glands.

Applicator fitted over alveolus and 10 platinum needles (·5 mm.), each containing 1·6 mc., and left in 7·7 days.

Radium collar 3 weeks later—6,800 mc.-hours.

J. T., age 76.—4 sq. cms. ulceration on under surface of anterior $\frac{1}{3}$, and involving alveolus.

Applicator fitted to irradiate alveolar involvement and ulcerated area on tongue. 10 needles distributed at spreading borders. 3 weeks later Radium collar—7,500 mc.-hours. 8 weeks later 12 needles buried in cervical glands. When last seen there was only one small palpable gland, but disease had spread all along alveolus and into tonsil area. The other 5 cases—3 on posterior $\frac{1}{3}$ and two on base of middle $\frac{1}{3}$—were treated on similar lines.

Results.

TOTAL DISAPPEARANCE OF PRIMARY GROWTH	4
PARTIAL DISAPPEARANCE OF PRIMARY GROWTH	2
DEATHS	2
DISAPPEARANCE OF CERVICAL GLANDS	0
IMPROVEMENT	6

Cervical Glands.

These were cases of glandular involvement secondary to surgical removal of growths in Buccal cavity and air-passages.

Dosages varying from 9,000–15,000 mc.-hours were administered.

Screenage, 2 mm. lead, at a distance of 3 cms. The time factor depended on quantity of Radon available.

In only one case can I confidently state that after treatment no gland could be palpated.

Conclusions.

(1) Secondary cervical glands are not radio-sensitive when compared with the primary growth.

(2) Complete surgical removal of gland-bearing area followed by external irradiation offers best hope for success.

Carcinoma of Breast, 4.

Keyne's technique was employed in 2 cases, in which the patients were relatively fat. In the remaining 2 the patients were very thin—not emaciated. In these cases the area in and around the tumour was needled, and over the rest of the area—*i.e.,* from tumour to above clavicle and into axilla—large surface dosages were given from a distance of 3 cms. Dosage varied up to 20,000 mc.-hrs. given in stages.

Results.

Complete Disappearance of Primary Growth	2
Partial Disappearance of Primary Growth	2
Complete Disappearance of Secondary Glands	2
Partial Disappearance of Secondary Glands	7
Recurrence of Secondary Glands	2

All cases were either border-line or inoperable.

Carcinoma of the Uterus.

The technique employed was:—

Intra-uterine irradiation.—24–30 millicuries, screened by 2 mm. lead in a thick gum-elastic tube. Time, 7·7 days, giving a dosage of up to 2,500 mc.-hours.

Irradiation of cervix and fornices.—Platinum needles, ·5 mm., and gold needles, ·5 mm., were buried all round the cervix and in lateral fornices. Ten to twelve needles, each containing 1·8 to 2 mc. each, were left in 7·7 days, giving a dosage of 1,800 to 2,000 millicurie-hours.

In one case it was impossible to give intra-uterine irradiation on account of the very advanced state of the growth. I hope later to give a second treatment, and that the uterine cavity will be accessible.

This case has only recently been treated. The other 3 were, when I heard of them last, progressing very favourably.

Sarcoma.

Two cases came for treatment. They were given massive doses over a wide area, and initial results are good, although one has had to have a second treatment.

Brain Tumours, 2.

Both these patients were treated subsequent to operation — for relief of pressure.

From a distance of 5 cms., screened by 2 mm. lead, a dose of 6,000 millicurie-hours was given in each case. Results were unsatisfactory, although there was some shrinking in one tumour.

Branchial Carcinoma, 1.

This was an early case, treated by surface application after surgical removal of growth.

10,000 millicurie-hours were administered in two stages, the interval being 7 weeks. After 10 months there is as yet no sign of recurrence, and the patient is very fit.

NON-MALIGNANT.

Warts, 10.

Technique varied from 10 millicuries applied for 15 minutes unscreened for small warts up to screened doses extending over 4–5 days. The results are very satisfactory.

Tuberculous Cervical Glands, 6.

Depending on the size of the area involved, doses up to 7,200 millicurie-hours on each side of the neck were administered, and usually in two stages.

Results have varied from complete disappearance to only a partial improvement. Two cases returned after 6–8 months with small recurrences.

Angiomata, 15.

The cavernous type are very satisfactory to deal with, and results are generally excellent. A dose of 600 millicurie-hours is given to the whole area—screenage, 2 mm. lead. Treatment is repeated every 6 weeks until the desired result is obtained. Number of treatments for each case vary from 6–10.

The "portional stain" is a much more delicate problem, and treatment must be carried out very carefully, because only very light screens are used, and placed directly on the skin. Some statistics claim 80 per cent. fading. I have not had many to treat. One at present under treatment is responding splendidly.

Keloids and Scars, 4.

Lengthy applications and heavily-screened doses offer remarkably satisfactory results when dealing with keloids. I find dense scars more difficult and refractory.

Lupus Vulgaris, 1.

The dose should be sufficiently large to produce a fairly marked reaction, and this I repeat in 8 weeks, if necessary. The case under review has had two applications, and at present I can see no active areas.

Hodgkin's Disease, 3.

One case was very far advanced—in a boy of 14. He had been surgically treated on two occasions before he came to Dublin. After the last operation, 6 months ago, the disease spread at an alarming rate. When first seen, in November, there were no glands except in the neck, and these were "huge."

Screened by 2 mm. lead at a distance of 2 cms., he got a dose of 7,400 millicurie-hours in 7·7 days.

Seen early in January, only two small glands remained, and these will be treated at once.

The other two cases were very early, and show no symptoms for the last 6 and 10 months, respectively.

Enlarged Spleen.

This case had an anæmia, probably specific in origin, with the spleen extending down to the umbilicus. The whole splenic area received a dose of 16,000 mc.-hours. The spleen retracted in size, but still remained palpable. It was since removed, and the patient is wonderfully fit.

1.	Malignant cases seen in year 1931 ...	....	...	... 99
2.	Do.	treated in year 1931	...	... 84
3.	Do.	do. by Surgery alone	...	... 0
4.	Do.	do. by Radium alone	...	... 63
5.	Do.	do. by X-rays alone ...	...	... 0
6.	Do.	do. by Surgery and Radium ...		... 21
7.	Do.	do. by Surgery and X-rays	...	... 0
8.	Do.	do. by Radium and X-rays	...	... 0
9.	Do.	do. by Surgery and Radium and X-rays		0

Report by C. Conor O'Malley, B.Sc., D.O.M.S.,

Central Hospital, Galway.

The following cases of cancer of the nose and throat and one eye case were treated only with Radon, with 1 mm. silver needles embedded in growth—Stevenson's method:—

1. T. S., male, age 64.—Extensive sloughing cancer of lower pole of tonsil extending on to floor of mouth.

7/1/'31, five (5) needles. Total dose, 480 mc.-hours.

Patient was not examined subsequent to leaving hospital. It was found impossible to give any information as to progress. Patient died 5 months later.

2. J. L., male, age 54.—Hard nodular growth, about the size of a hen's egg, projecting from left canine fossa area. History of small growth present for a long time, which had recently commenced to enlarge rapidly. The growth looked definitely malignant.

26/1/'31, four (4) needles. Total dose, 245 mc.-hours. Patient was not examined subsequent to leaving hospital. A letter from him on 1/3/'32, over one year after treatment, says that the tumour has completely disappeared.

3. M. K., male, age 61.—Patient first seen in January, 1931. There was a fleshy black wing-shaped growth extending from the left inner canthus outwards, and partially covering the limbus. There were numerous small jet-black mushroom-shaped growths extending along the upper and lower fornices, and also on the inner surfaces of the lids. I removed the growth from the inner canthus, and the pathologist's report was melanotic epithelioma. There was no involvement of the globe. Fundus and media normal, and vision 6/6ths.

4/2/'21, one (1) needle inserted just under the conjunctiva in the lower fornix. After 12 hours the needle was removed, and inserted similarly into the upper fornix for 12 hours. Total dose, 192 mc.-hours. Patient was examined on 27/2/'32, one year later. The growth has completely disappeared. The eyeball is freely mobile, one small adhesion below. Fundus and media normal, and vision 6/6ths.

This was an interesting case, owing to the rare type of growth, and the absence of any damage to the eye by irradiation.

4. A. L., male, age 63.—Epithelioma on palate and tonsil. History indefinite.

11/4/'31, four (4) needles embedded. Total dose, 405 mc.-hours.

This patient was not re-examined after leaving hospital, and failed to answer several letters of inquiry. It is ascertained, however, that he is still alive, and apparently carrying on.

5. T. W., male, age 65.—Extensive cancer of tongue and floor of mouth. Advanced cachexea. 19/5/'31, needles inserted. Total dose, 480 mc.-hours.

The patient was not examined subsequent to leaving hospital, and there is no note on progress. A communication from his doctor said that he had died three months later.

6. M. O., female, age 58.—This patient was sent to hospital with the diagnosis of an orbital abscess.

The right eye was proptosed up and out. There was free hæmorrhage from the nose, due to a growth in the right ethmoid, which was extending nasally and orbitally. 17/9/'31, one needle was embedded in the tumour of the nose, and one needle in the orbit. Total dose, 540 mc.-hours. There was some reaction. The nasal hæmorrhages ceased, and did not give any further trouble. This patient was not examined subsequent to leaving hospital. On inquiry, it was found that she had further radium treatment, and had died in February, 1932.

7. J. H., male, age 60.—Epithelioma on hard palate on left side, with a deep sloughing ulcer. 1/10/'31, three (3) needles inserted. Total dose, 305 mc.-hours. 20/11/'31, patient came up for examination. The growth had entirely disappeared. Patient feels well, and has no discomfort.

8. D. C., age 68.—Extensive sloughing epithelioma or palate and left tonsil extending to and involving left side of epiglottis. There was a large swelling in the cervical region on the left side, probably mainly due to a septic inflammation.

21/10/'31, four needles placed in growth. Total dose, 375·5 mc.-hours. 10/2/'32, patient came up for examination. The growth has completely disappeared, and there is no trace of swelling in the cervical region.

There is a small slough at the upper pole of tonsil, apparently due to an overdose at this point. Apart from some discomfort due to the slough, patient feels well and eats well.

One can say with a certain amount of confidence that Radon does well in cancer of the palate and tonsil and tonsil pillars, even when the growth is extending on to the tongue. On the other hand, the results on intra-nasal growths are not so satisfactory.

I wish to thank the R.D.S. Radon Staff for their courtesy and promptness in supplying Radon.

REPORT BY BETHEL SOLOMONS, M.D., F.R.C.P.I.,

Master, Rotunda Hospital.

Ten patients had radium at the Rotunda during the year. Seven were for carcinoma of the cervix, and one of these died a couple of days after the second application, without any apparent cause. One patient had cervical and vaginal carcinoma, and one was treated for secondary growths in the vagina.

The tenth case was an unmarried girl, who was treated for heavy menstruation.

REPORT BY SIR ROBERT WOODS, M.B., M.CH., F.R.C.S.I.

The radium I have obtained from the Institute has only been used in nasal cases, and they have been confined to three diseases, namely—sarcoma, malignant granuloma, and tubercular disease.

As far as the sarcoma is concerned, X-rays were used also, and it is not possible to say exactly what part each played.

In the malignant granuloma the effect was to repress new manifestations of the disease, and in the case of the tuberculosis it was simply used as a mild caustic.

REPORT OF MALIGNANT CASES TREATED AT THE CITY OF DUBLIN SKIN AND CANCER HOSPITAL DURING THE YEAR 1931.

Number of cases of all types of malignant disease:—

1.	Number seen during 1931	171
2.	Number treated during 1931	171
3.	Do. by Surgery alone	3
4.	Do. by Radium alone	2
5.	Do. by X-ray alone	106
6.	Do. by Surgery and Radium ...	1
7.	Do. by Surgery and X-rays ...	8
8.	Do. by Radium and X-rays ...	50
9.	Do. by Surgery, Radium, and X-rays	1

No. 22.

THE INHIBITION OF CHEMICAL REACTIONS. PART 5*. THE INFLUENCE OF PYRIDINE AND OTHER SUBSTANCES ON THE ABSORPTION OF ETHYLENE BY SULPHURIC ACID, AND ON THE SURFACE TENSION OF SULPHURIC ACID.

By WILLIAM SYDNEY EAGAR HICKSON, M.Sc.,

AND

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Fellow of Trinity College, Dublin.

[Read February 23. Published separately June 15, 1932.]

Examples of negative catalysis (the inhibition of chemical reactions) may be divided roughly into two classes: (*a*) reactions in which the inhibitor is present in quantity comparable to the reagents; and (*b*) those in which the inhibitor is present in very minute amount, one part of the inhibitor in a million of reacting substance sometimes having an appreciable effect.

Two principal theories have been advanced to account for reactions of type (*b*), which may be called cases of "trace inhibition." The first is based on the Chain Theory of Christiansen and Bäckström, according to which a product of the original molecular reaction possesses sufficient energy to "activate" another molecule, and thus initiate a second reaction, and a product of this second reaction is sufficiently "active" to set up a third reaction, and so on. The absorption of a single quantum of energy may thus result in the reaction of many thousands of molecules. The inhibitor acts by intercepting an "active" molecule and deactivating it in some way. It is clear that a small proportion of inhibitor cannot be expected to produce a large effect, unless the chain, in the absence of inhibitors, consists of a great number of links, or the life of the active molecule is unusually long. The probability of a molecule of inhibitor, present in small concentration, encountering the energy-bearing "link" will be small if the total lifetime of the chain is short.

The second theory is the Surface Theory. According to this, the reaction

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* Parts 1–4 have been published in the Journal of the Chemical Society (1928, 1204; 1928, 3256; 1930, 104; 1931, 420).

is considered to take place on a solid surface (usually the surface of the containing vessel). The inhibitor owes its efficiency to the fact that, although present in relatively small concentration in the reaction mixture as a whole, it is preferentially adsorbed at the surface, which is the site of reaction, and its concentration in the place where it is most likely to be effective is not small, but large.

One of the writers has suggested, in Parts 3 and 4 of this series (Bailey, J. Chem. Soc., 1930, 104; Bailey and ffrench, *ib.*, 1931, 420), that the efficacy of certain inhibitors, such as sulphur, in preventing the oxidation of benzaldehyde by atmospheric oxygen is due to concentration of the inhibitor at a surface, but on this occasion at a liquid surface, the benzaldehyde-air interface.

Davis and Schuler (J. Amer. Chem. Soc. (1930), **52**, 721) and Davis and Crandall (*ib.* (1930), **52**, 3769) have shown that when ethylene is absorbed by sulphuric acid of a given concentration the velocity of absorption depends only on the area of the liquid surface and the concentration of the gas. When the sulphuric acid was stirred beneath the surface by a special stirrer, which could revolve without directly agitating the surface, the absorption rate was not affected as long as the revolutions of the stirrer did not exceed 480 per minute. The reaction of the ethylene with sulphuric acid apparently took place entirely in the thin "stationary film" which exists at the surface of even well-stirred liquids. The thickness of this film has been shown, in the case of water at room temperature, to vary in depth from 0·04 cm. when quiescent to 0·0045 cm. when the water is stirred underneath the surface at 1,000 revolutions per minute (Lewis and Whitman, Ind. Eng. Chem. (1924), **16**, 1215; Davis and Crandall, J. Amer. Chem. Soc. (1930), **52**, 3759). Even a film of the latter thickness, as pointed out by Davis and Crandall, is over 100,000 times the diameter of the water molecule, and the stationary film, in the case of sulphuric acid, is doubtless of the same order of thickness.

The experimental evidence does not show whether the ethylene reacts with sulphuric acid throughout the stationary film or in a layer a few molecules deep at the actual surface, but the latter seems more probable; for, if ethylene were sufficiently soluble in sulphuric acid, and at the same time sufficiently unreactive for molecules of ethylene to penetrate into the liquid to a depth of some thousands of molecules, there seems no reason why some of the gas should not penetrate more deeply, and react in the body of the liquid. This, according to the results of Davis and Schuler, does not seem to be the case, inasmuch as agitation of the bulk of the liquid does not affect rate of absorption.

Since, then, we seem to have here a chemical reaction which takes place entirely at, or near, a gas-liquid interface, we should also have the possibility of getting a negative catalysis of the type postulated in the work of autoxidation of benzaldehyde, in which the inhibitor is considered to operate at the gas-liquid interface. If the inhibitor were preferentially adsorbed at this interface, then a small total concentration might maintain a sufficiently high concentration at the liquid surface to provide an example of "trace inhibition."

It has been shown by Lebeau and Damiens (Comptes Rendus (1913), **156**, 557), Damiens (*ib.* (1922), **175**, 585), Gluud and Schneider (Ber. (1924), **57 B**, 254), and Lommel and Engelhardt (*ib.* (1924), **57 B**, 848), that V_2O_5, U_3O_8, sulphotungstic and sulphomolybdic acids, ferric ammonium alum, and the sulphates of lead, calcium, iron, copper, mercury, and silver, act as positive catalysts for the interaction of ethylene and sulphuric acid, but only water and benzene, so far as the writers are aware, have been noted as reducing the reaction velocity (Damiens, l.c.).

Apparatus and Method of Calculation.

The apparatus consisted of four glass flasks of about 100 ml. capacity, with ground-glass stoppers, connected by all-glass connections to (*a*) manometer tubes, (*b*) a Geryk pump, and (*c*) a cylinder of compressed ethylene. The taps were so arranged that the ethylene could be admitted simultaneously to all four flasks. One flask contained about 50 ml. of sulphuric acid of a given strength, and the others a similar volume of the same acid containing known concentrations of the inhibitor under investigation. In all cases, the walls of the flask were covered, by shaking, with sulphuric acid, and allowed to drain for 24 hours before the ethylene was admitted. This procedure was found to give practically the same absorption rate as when the sulphuric acid was introduced so carefully that no splashing of the walls occurred. Presumably, no sulphuric acid remained on the walls after 24 hours. The object of this procedure was to avoid inaccuracies arising from unintentional splashes of acid on the vessel walls.

The volumes of the flasks and their connecting tubes were measured in the following way :—Let x and y ml. be the volumes of flasks 1 and 2 respectively. 50 ml. of sulphuric acid were placed in each flask, and flask 2 was exhausted, while flask 1 contained air at atmospheric pressure. The tap connecting the two flasks was opened, and the resulting pressure noted.

$$\frac{x + y - 100}{x - 50} = \frac{\text{initial pressure}}{\text{final pressure}}.$$

The acid was then removed from 2, the flask exhausted, and the operation repeated. In this case,

$$\frac{x + y - 50}{x - 50} = \frac{\text{initial pressure}}{\text{final pressure}}.$$

From these two equations, the volume of each flask may be deduced. The volume in each case was about 150 ml., of which nearly 50 ml. was accounted for by the connecting tubes. The sulphuric acid (50 ml.) reached almost to the widest part of the flask, and a slight variation in volume of sulphuric acid

used made, therefore, very little difference in the area of the liquid surface. Allowance for these small variations was, however, made.

Number of flask.	Total volume.	Area of liquid surface, when 50 ml. acid present.
1.	148·0 ml.	28·8 sq. cms.
2.	145·2 „	27·0 „
3.	147·1 „	28·4 „
3 a.	154·7 „	27·1 „
4.	149·3 „	28·4 „

The volume of ethylene was not measured before admission to the flasks, and the absorption of ethylene during the first few seconds was, therefore, not registered. Davis and Schuler, however, (l.c.) have shown, by experiments in which the volume of ethylene was measured before admission to the reaction vessel, that there is no rapid initial absorption, such as might be due to physical solution of the gas in the liquid.

Sulphuric acids of different strengths were prepared by mixing appropriate quantities of pure fuming sulphuric acid and strong aqueous sulphuric acid (94 per cent. H_2SO_4). The strength of each batch of acid was estimated by diluting a weighed quantity to a litre with water, and using the resultant solution to titrate a known weight of sodium carbonate, freshly prepared by ignition of the pure bicarbonate. The ethylene used was obtained from the British Oxygen Company. The pyridine employed in the final research was carefully purified by drying over potash and subsequent fractionation.

Sulphuric acid was always present in overwhelmingly greater quantity than was stoichiometrically necessary.

Davis and Schuler found that, for a given area of acid, the reaction was monomolecular with respect to ethylene, and this conclusion is borne out by the present work. The reaction was carried out at constant volume, and followed by the decrease in ethylene pressure.

$$-\frac{dP}{dt} = K \,.\, P,$$

$$\therefore K = \frac{1}{t} \,.\, \log_e \frac{P}{P'},$$

where t is the interval in seconds between two readings, and P and P' are the pressures in mm. of mercury at the beginning and end of the interval.

Davis and Schuler calculated a specific absorption coefficient, C, using the equation,

$$C = K \frac{\text{Total volume of gas}}{\text{Total area of absorbing surface}}.$$

C they define as "the proportional change in volume at constant pressure which would take place in an infinitesimally short time when 1 c.c. of the gas

was in contact with 1 sq. cm. of an acid surface having the average absorbing power of the total acid surfaces . . . At constant pressure, C is evidently equal to the average number ml. of gas absorbed by 1 sq. cm. of acid surface in 1 second."

A similar method of calculation has been adopted in the present paper.

As Common Logarithms have been employed, and the minute taken as unit of time, K and C should be multiplied by $\frac{2 \cdot 3 0 3}{6 0}$ to obtain values corresponding to those of Davis and Schuler.

Preliminary Experiments.

It has long been known that strong sulphuric acid absorbs ethylene more rapidly than the dilute acid. Water is, therefore, an inhibitor. Preliminary experiments were made to discover other substances which would retard absorption.

$6{\cdot}25 \times 10^{-3}$ mols of each substance were added to 50 ml. of acid (94·2 per cent. H_2SO_4 : sp. gr. 1·837). Of this acid, 91·8 gms. (50 ml.) contained the equivalent of 87·4 gms. of H_2SO_4. If we regard the water and pyridine molecules as free from combination with H_2SO_4 for the purpose of calculation, the concentration of inhibitor is about 5·3 molecules in every 1,000 molecules of total liquid.

Parallel experiments with plain H_2SO_4 and H_2SO_4 containing inhibitor were performed at air temperature (about 10° C.).

Let K = reaction constant for plain H_2SO_4;

Let K' = reaction constant for H_2SO_4 containing inhibitor.

TABLE 1.

Added substance.	$\frac{100\,K'}{K}$
Naphthalene	68·7
Phenol	82·6
Piperidine	84·5
m-Cresol	86·2
Pyridine	87·0
Acetone	87·7
Ammonium Sulphate	91·9
Quinoline	93·2
Acetamide	94·1
Benzene	95·3
Aniline	95·6
Water	98·1
Chloroform	103·1

These preliminary experiments, although not laying claim to a high degree of accuracy, made it clear that all the above substances, except chloroform, inhibit the reaction, and this was confirmed by additional experiments in the cases of naphthalene, phenol, pyridine, and water.

It was finally decided to make a more exhaustive examination of the effect of (*a*) water, (*b*) sulphur trioxide, (*c*) pyridine.

Final Experiments.

Four series of experiments were carried out:—

A. The effect of water as inhibitor.

B. The effect of sulphur trioxide as accelerator.

D. The effect of pyridine as inhibitor in H_2SO_4 containing 0·3 mol of water per litre (99·46 per cent. H_2SO_4).

E. The effect of pyridine as inhibitor in fuming acid containing 2·65 mols of sulphur trioxide per litre.

A number of experiments with various strengths of sulphuric acid, with and without pyridine, showed that the Temperature Coefficient for a rise of 10° was approximately 1·63. This has been used in correcting the value of C to 13° C.

TABLE 2.

Example of an individual reaction (Series D).

Flask 2.— Vol. of liquid, 53·2 ml.; vol. of gas, 92·0 ml.; area of liquid surface, 27·4 sq. cms.; temperature, 13·5° C.

Water, 0·3 mol per litre; pyridine, 1·17 mols per litre. K and C are as defined, being in each case 30 or 35 minutes.

Time (mins.).	Pressure (mm. *Hg*).	$K \times 10^3$.
0	770	
10	752	
20	735	
25	726	
30	717	
40	702	1·00
50	687	0·98
60	670	0·98
70	657	0·96
85	635	0·98
100	616	0·93
115	595	0·94
130	575	1·00
150	549	1·00

Mean $K \times 10^3 = 0{\cdot}97$; $C \times 10^3 = 3{\cdot}26$ at 13·5° $= 3{\cdot}18$ at 13°.

TABLE 3.

Summary of experimental results.

A. Water as inhibitor. B. Sulphur trioxide as accelerator. D. Pyridine as inhibitor (molar conc. of water = 0·3). E. Pyridine as inhibitor in fuming acid (molar conc. of SO_3 = 2·65).

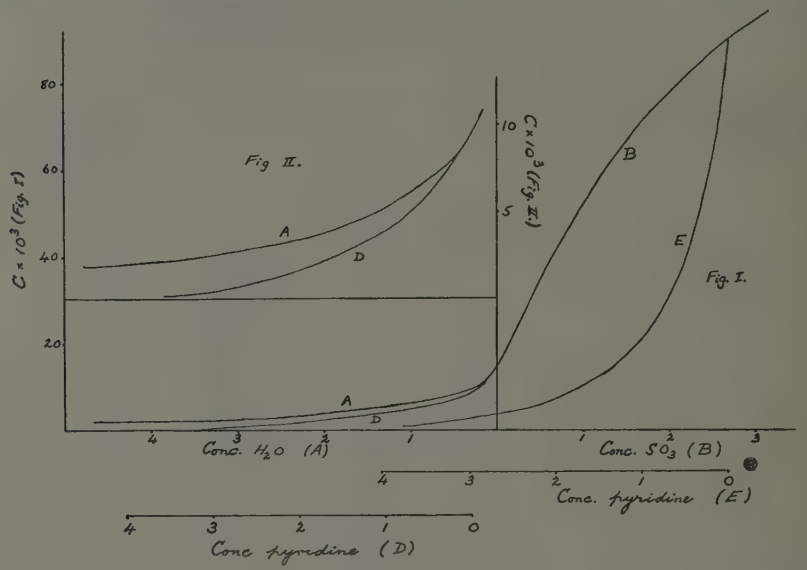
A		B		D		E	
Conc. water (mols p. l.).	C. × 10^3.	Conc. SO_3 (mols p. l.).	C. × 10^3.	Conc. pyridine (mols p. l.).	C. × 10^3.	Conc. pyridine (mols p. l.).	C. × 10^3,
0·11	11·23	0·05	15·0	0·00	8·86	0·00	89·1
0·32	8·57	0·22	22·9	0·13	7·49	0·13	76·1
0·65	7·61	0·44	37·5	0·25	7·17	0·25	61·3
0·86	6·40	1·00	51·7	0·49	6·22	0·37	53·1
1·18	5·22	1·80	72·0	0·73	4·57	0·49	42·4
1·71	4·30	2·15	77·2	0·95	3·81	0·61	37·3
2·57	3·34	2·39	77·7	1·17	3·18	0·73	31·7
3·11	2·93	2·74	92·5	1·38	2·78	0·84	26·2
3·64	2·29	3·09	95·6	2·20	1·20	0·96	23·3
4·69	1·65			2·66	0·70	1·07	18·3
				3·07	0·37	1·28	16·0
				3·49	0·20	2·04	7·87
						2·48	5·32
						2·60	4·19
						3·11	2·42
						3·42	2·42
						3·75	0·96

The results of *A*, *B*, and *E* have been plotted in Figure 1, where it will be seen that sulphur trioxide has a very marked effect in accelerating the reaction, and that the inhibitory effect of pyridine in fuming sulphuric acid is very great. In Figure 2, *A* and *D* are plotted, and it is clear that the inhibiting effect of pyridine in ordinary sulphuric acid is much greater than that of water.

If the inhibiting or accelerating substance were preferentially adsorbed at the gas-liquid interface, one would expect that a unimolecular layer of solute would be formed at the surface while the concentration in the body of the liquid was still moderate. If a substance thus adsorbed inhibited or accelerated a reaction which normally took place at the surface, a sudden discontinuity should occur in the curve (reaction velocity–inhibitor concentration) at the point at

which the unimolecular layer of solute is completed. In the curves of Figures 1 and 2 no signs of discontinuity are to be seen.

Either (*a*) the reaction does not take place in the surface layer, or (*b*) the solute is not preferentially adsorbed at the interface. In view of the evidence of Davis and his collaborators, (*a*) seems unlikely. The alternative (*b*) can be



FIGS. 1 AND 2.

tested by measuring the surface tensions of solutions of water, sulphur trioxide, and pyridine in sulphuric acid, and this was undertaken.

Measurements of Surface Tension.

These measurements were made by the drop-weight method, using a simple form of apparatus, made entirely of glass, to avoid possible action of the acid on any metal which might be employed. It consisted of a vertical tube, into which the acid was introduced at the commencement of each determination, the initial level being the same in every case. The upper extremity was protected by a guard tube, to prevent the entry of water vapour during the determination; the lower terminated in a thick-walled capillary tip, with a carefully ground, approximately circular, end. The mean external diameter of the end was 6·04 mm., the extremes being 5·95 mm. and 6·17 mm. This tip was sealed into a short, wide glass tube, the end of which was ground to receive a weighing-bottle. The capillary tip projected into this tube to a distance of about 2 cm. The whole apparatus, almost to the point of attachment of the weighing-bottle, was enclosed in a water-jacket, so as to secure constancy of temperature. The drops formed

under the influence of gravity alone, but, as the capillary was very fine, the time of drop formation was long.

The simple equation, used by early workers, $W = 2\pi r\sigma$ (where W = the weight of the drop, r = the radius of the tip, and σ = the surface tension) would be true only if the pendant drop were supported entirely by the surface tension of the surface film at the edge of the tip, and if the entire drop fell. This is not the case.

It has been shown by Lohnstein (Ann. Physik (1906), [4], **20**, 237, 606; Z. physik. Chem. (1908), **64**, 686; (1913), **84**, 410), Harkins and Humphery (J. Amer. Chem. Soc. (1916), **38**, 228), and Harkins and Brown (*ib.* (1919), **41**, 499) that a more exact equation is

$$W = 2\pi r\sigma \cdot f\left(\frac{r}{l}\right)$$

(where l is a linear dimension of the drop), and that, as the cube root of the volume of the drop, $V^{\frac{1}{3}}$, varies as a linear dimension of the drop, the equation

$$W = 2\pi r\sigma \cdot f'\left(\frac{r}{V^{\frac{1}{3}}}\right)$$

may be used.

The values of this latter function determined by Harkins and Brown (l.c., 519) have been used in this paper (column 7 of Table 4).

The theory of Lohnstein demands that the drop shall detach itself infinitely slowly. This is, of course, not the case in practice, and more elaborate apparatus has been devised by Harkins and Brown (J. Amer. Chem. Soc. (1916), **38**, 246, and l.c.) to ensure that the drop detach itself as slowly as possible.

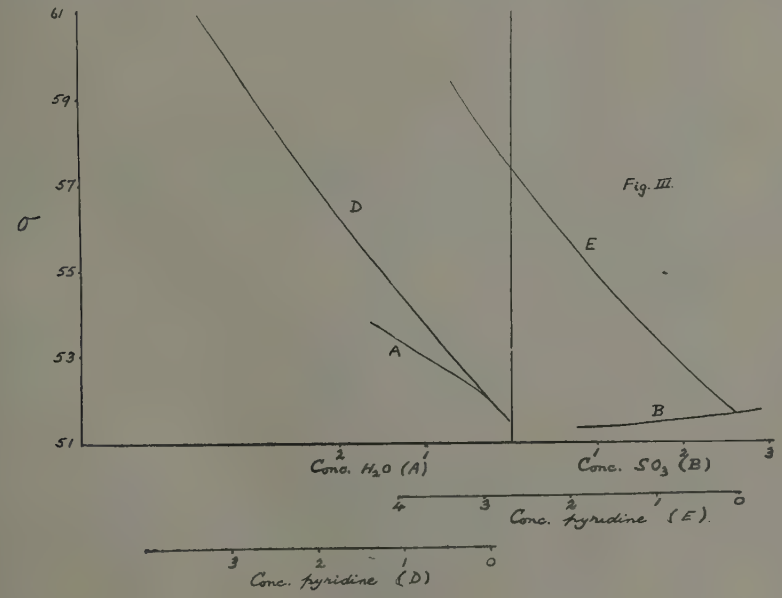


FIG. 3.

A small, constant error is introduced in the present work, in consequence of the slightly more rapid detachment of the drop, but, as the conditions were the same throughout, the comparative value of the results should be unimpaired.

In order to correct for errors of evaporation, two experiments were performed in each estimation, seven drops being weighed in the first and thirty-seven in the second. The difference between the two weighings, divided by thirty, was taken as the weight of one drop, W.

The temperature at which the surface tension was measured varied from 10·5° to 14·2°. It is clear, however, from the work of Morgan and Davis (J. Amer. Chem. Soc. (1916), **38**, 555) that the effect of small changes in temperature on the surface tension of strong sulphuric acid is so small as to make it unnecessary to apply any correction.

The experimental results are shown in Table 4, and plotted in Figure 3.

The results for sulphuric acid containing water and sulphur trioxide give curves resembling those obtained by Morgan and Davis (l.c.), but σ is about 1·3 dynes per cm. higher, a discrepancy possibly to be accounted for by the constant error referred to above.

TABLE 4.

Surface Tensions.

A.—*Water in Sulphuric Acid.*

Temp. (°C.).	Conc. of water (mols p. l.).	Wt. of drop $W \times 10^2$ (gms.).	Spec. gravity.	Vol. of drop $V \times 10^2$ (mls.)	$\frac{r}{V^{\frac{1}{3}}}$	$f'\left(\frac{r}{V^{\frac{1}{3}}}\right)$	σ (dynes per cm.).
13·4	0·11	6·030	1·839	3·280	0·9435	0·6028	51·73
12·4	0·65	6·126	1·840	3·330	0·9386	0·6023	52·60
13·1	1·18	6·192	1·840	3·365	0·9354	0·6020	53·20
12·4	1·71	6·265	1·841	3·403	0·9320	0·6017	53·85

B.—*Sulphur Trioxide in Sulphuric Acid.*

	Conc. of SO_3.						
13·6	1·00	5·989	1·859	3·222	0·9490	0·6034	51·33
13·5	1·34	5·999	1·864	3·219	0·9492	0·6034	51·42
12·6	1·57	6·003	1·867	3·215	0·9496	0·6034	51·45
12·4	2·74	6·028	1·885	3·198	0·9515	0·6035	51·65

D.—*Pyridine in Aqueous Acid* (*Molar conc. of water* = 0·3).

	Conc. of pyridine.						
11·1	0·00	6·070	1·84	3·300	0·9414	0·6025	52·06
10·5	0·82	6·289	1·79	3·513	0·9222	0·6003	53·91
11·5	1·90	6·622	1·74	3·807	0·8976	0·5997	57·08
11·0	2·66	6·858	1·70	4·032	0·8806	0·5994	59·12
10·8	3·36	7·050	1·67	4·222	0·8674	0·5993	60·80

E.—*Pyridine in Fuming Acid* (*Molar conc. of sulphur trioxide* = 2·65).

13·8	0·37	6·080	1·862	3·266	0·9446	0·6028	52·17
13·9	0·73	6·149	1·844	3·335	0·9382	0·6023	52·80
13·9	1·07	6·225	1·829	3·402	0·9320	0·6017	53·51
14·0	2·04	6·488	1·775	3·655	0·9100	0·6003	55·90
14·0	2·60	6·630	1·730	3·832	0·8958	0·5997	57·18
14·2	3·38	6·885	1·680	4·099	0·8758	0·5993	59·41

The effect of a molecule of pyridine in increasing the surface tension is greater than that of a molecule of water, and much greater than that of a molecule of sulphur trioxide. The increase in surface tension is approximately proportional to the concentration of pyridine, and the effect of addition of a given weight of pyridine is much the same, whether the pyridine is added to fuming or to slightly aqueous acid.

Interpretation of Results.

Since surface energy tends towards a minimum, a solute which lowers the surface tension of a solvent will accumulate at the surface; conversely, when a solute raises the surface tension, its concentration in the surface layer is less than in the body of the liquid.

Attempts to predict exactly the excess or deficiency of solute (Γ) in the surface layer have been made, using the "simplified Gibbs equation,"

$$\Gamma = -\frac{c}{RT} \cdot \frac{d\sigma}{dc},$$

where c is the concentration of solute, R the gas constant, T the absolute temperature, and σ the surface tension. This equation is admittedly inexact, and is brought more into accord with theory by substituting "activity" for "concentration."

In many cases, as in the present, the activities are not known, and the work of McBain and Davies (J. Amer. Chem. Soc. (1927), **49**, 2230), and McBain and Du Bois (*ib.* (1929), **51**, 3534), in which direct determinations of Γ are made, casts doubt on whether Γ can be estimated by means of this equation, even if activities are known and used. McBain and Davies (l.c.) point out that the equation ignores such factors as electrical effects and difference in composition of the adsorbed solute from the free solute.

These investigators have shown that the excess solute in the surface layer is greater than that predicted by the Gibbs equation in the cases of aqueous solutions of amyl alcohol, acetic, butyric, caproic, and nonylic acids, phenol, *p*-toluidine, resorcinol, thymol, and camphor (all of which lower the surface tension of water), and the deficiency greater than that predicted in the case of sodium chloride (which raises the surface tension). Results similar to McBain's for solutes lowering the surface tension were obtained by Bailey and ffrench (J. Chem. Soc., 1931, 420) for solutions of *iso*propyl and *sec.* butyl alcohols in water.

Any attempt to calculate the actual concentrations, in the present case, of water, sulphur trioxide, and pyridine, in the surface layer of their solutions in sulphuric acid, would clearly be futile; but, since it has never been disputed that the Gibbs equation gives a result of the right *sign*, we may safely assume

that the concentration of these solutes in the surface layer is always smaller than in the bulk of the liquid, since they cause a rise in surface tension.

It is inconceivable, however, that, even if a solute raises the surface tension very much, the surface layer can ever be entirely free from molecules of the solute. The surface concentration will, moreover, increase with the bulk concentration, and it seems likely that at low dilutions the one will be roughly proportional to the other, unless, indeed, there is a difference in composition between the adsorbed and the free solute. In the case of a solute which raises the surface tension, the establishment of a unimolecular layer of solute at the surface is, of course, out of the question.

If we are right in assuming, on the grounds already stated, that the ethylene-sulphuric acid reaction takes place in the surface film, we can now understand why the inhibition curves (*a*) show no sudden discontinuity, and (*b*) exhibit no "trace inhibition."

It is clear from these curves that the inhibiting effect of the first small portion of pyridine or water added is greater than that of subsequent additions, and this might seem in contradiction to the suggestion just made that, at low dilutions, the surface concentration of inhibitor will probably be roughly proportional to the bulk concentration. No real contradiction exists. Each inhibitor molecule may be assumed to have a "sphere of influence," which will be measured by the number of molecules with which it would collide in the time taken for molecules of ethylene and sulphuric acid to complete their reaction. If the surface concentration of inhibitor molecules is doubled, the number of reagent molecules brought within the sphere of influence of inhibitor molecules will not be doubled, as some reagent molecules will be included in two or more spheres of influence. The inhibition effect in the surface layer must, therefore, increase more slowly than the inhibitor concentration.

If a substance can be discovered which will (*a*) reduce the surface tension of sulphuric acid, and (*b*) inhibit absorption of ethylene, "trace inhibition" will probably be found to occur, and the reaction curve should exhibit discontinuity.

It is interesting to observe that, where pyridine and water are present in the bulk in equal molecular concentrations, the pyridine, although probably present in the surface layer in lower molecular concentration than water (inasmuch as it causes a greater rise in surface tension), is a more powerful inhibitor. Pyridine must, therefore, exert a much greater specific effect than water.

Summary.

1. The absorption of ethylene by sulphuric acid is inhibited by naphthalene, phenol, piperidine, *m*-cresol, pyridine, acetone, ammonium sulphate, quinoline, acetamide, benzene, aniline, and water, and accelerated by chloroform and sulphur trioxide.

2. The inhibition by pyridine and water, and the acceleration by sulphur trioxide, have been investigated in detail. The resulting curves show no discontinuities.

3. The surface tensions of sulphuric acid solutions of pyridine, water, and sulphur trioxide have been determined.

4. It is shown that the results are consistent with the theory that inhibition takes place in the surface layer of the liquid.

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No. 23.

A METHOD FOR AUTOMATICALLY RECORDING THE OXYGEN INTAKE OF LIVING TISSUES.

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[Communicated by Professor H. H. Dixon.]

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Introduction.

The number of methods which have been described for the determination of both oxygen intake and carbon dioxide output of living tissues is very considerable. No single one, however, is entirely suitable for the investigation of the particular problem at which the writer has been working. Briefly, some of the desiderata of the "ideal" apparatus are as follows: the plant must be surrounded by a desired gas mixture (such as air), the composition of which must not change appreciably during the course of the experiment. The carbon dioxide concentration must be very low as in ordinary air. The apparatus must also be capable of rapidly responding to changes in the rates of oxygen intake and carbon dioxide output by the tissue.

These conditions are not realised when eudiometric methods are used. Such methods have been used in almost all the investigations of the respiratory quotients and rates of oxygen intake of plants (*cf.* 4). Numerous modifications of the method have been devised, but all of them depend on enclosing the plant in a vessel and determining the change in composition of the gas mixture in the vessel. Godwin's method (3) consists of leading a very slow current of air over the respiring plant and determining the composition of the outflowing air. The method is, however, somewhat laborious, and rapid changes in the rate of oxygen intake could not be detected by its use.

The method of Fernandes (2), which has been used later in a slightly modified condition by Wetzel (5), does, however, practically fulfil nearly all the required conditions.

In a somewhat different modified form it has been used by the writer for some years. It has, however, been found possible recently greatly to simplify the apparatus and to make it self-recording. So far as the writer is aware no method has been described capable of recording automatically the changing rates of oxygen intake of plant tissues which are observed when they are subjected to continued periods of starvation in darkness. It therefore seems

desirable to describe briefly the method used and the automatic recording arrangement.

The principle is based on that of Fernandes' apparatus, which consisted of a closed system round which the enclosed air was caused to circulate by means of a pump: the air passed over the plant material, and the carbon dioxide carried by it from the respiring plants was absorbed in tubes filled with baryta: the oxygen taken in by the plant tended to cause the volume of the air in the apparatus to decrease, but this decrease was prevented by the constant addition of oxygen electrolytically. The volume of added oxygen was obtained by measuring the volume of hydrogen simultaneously evolved from the other electrode. The rate of addition of oxygen was controlled by the experimenter so that it remained equal to the rate at which it was being absorbed by the plant. The composition of the air surrounding the plant was thus kept constant. It was necessary that the experimenter should be constantly beside the apparatus adjusting the rate of oxygen supply to the required amount. This clearly is not possible where the experiments are continued for several days.

Construction of the Apparatus.

The modified form of the apparatus differs in many details from Fernandes' apparatus. Air circulates round a closed system consisting of the pump, *k* (fig. 1), the plant chamber, *j*, and the "Pettenkofer" tube, *l*, which

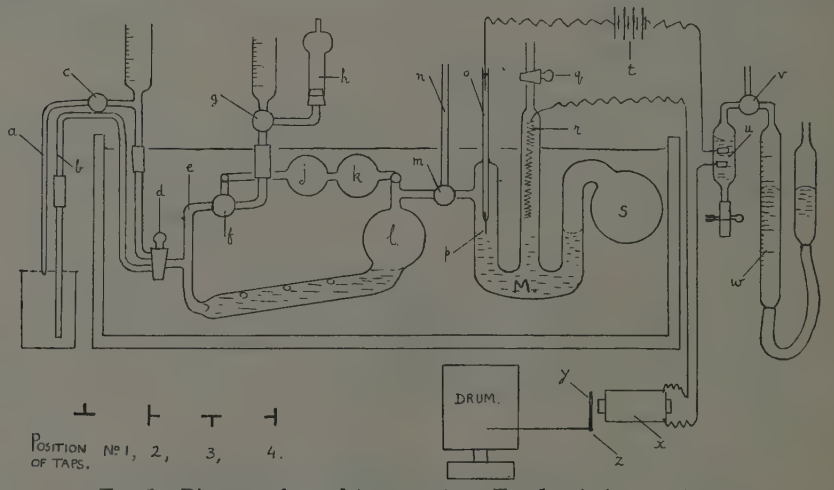

Fig. 1.—Diagram of complete apparatus. For description, see text.

is filled with baryta (the tap *f* is placed in position 4). This system is joined by means of the manometer, *M*, to the closed space, *s*, the volume of which approximately equals that of the enclosed spaces $j + k + l$.

The apparatus is a modified differential manometer; changes of temperature or of atmospheric pressure therefore cause no change in the level of the liquid in the manometer tube, *M*. Any changes in the level of this liquid are due to changes in the volume of gas in the enclosed system $j + k + l$. Such changes are brought about by the respiratory exchanges (oxygen absorption) of the tissue in the plant chamber.

The volume of air (and incidentally its composition) is kept constant automatically by the following device. A platinum electrode, *p*, is fused into the left-hand limb of the manometer just above the level of the liquid: a second electrode, *r*, makes contact with the liquid in the manometer; this liquid is a solution of copper sulphate containing copper hydroxide in suspension. When the plant absorbs oxygen, the level of the copper sulphate solution rises in the left-hand limb of the manometer and makes contact with the platinum electrode, whereupon a current starts flowing, and oxygen is added to the part of the apparatus containing the plants. The level of the liquid consequently falls again, and contact with the platinum electrode is broken and the oxygen supply is cut off. As sulphuric acid is also formed at the platinum electrode, copper hydroxide is suspended in the copper sulphate solution, and, by neutralising the sulphuric acid, this prevents the formation of hydrogen at the other electrode, *r*. Copper is deposited on this electrode.

A pair of platinum electrodes, *u* (fig. 1), immersed in baryta solution are joined in series with the electrodes, *p* and *r*. The volume of oxygen supplied to the plant at *p* is, clearly, one-third of the volume of oxygen + hydrogen evolved at *u* (at same temperature and pressure); this volume is measured by the gas burette. This device, therefore, secures the automatic addition of oxygen at the same rate as the plant removes it. It is to be noted, moreover, that it is a marked improvement over the method of Fernandes in that its accuracy is quite unaffected by changes of atmospheric pressure, since the whole apparatus is totally enclosed. Changes of atmospheric pressure may introduce considerable errors into the Fernandes method (*cf.* 4). The automatic control of the rate of addition of oxygen is, of course, also a new feature.

It remains now to describe shortly the technical details of the construction of the apparatus and of the self-recording mechanism.

The apparatus consists of three separate parts, the pump, plant chamber, and the CO_2-absorbing vessel + manometer + compensator, which are joined to each other by rubber tubing. The whole is immersed in a water-bath kept at a constant temperature.

The pump.—The gas circulating pump proved to be the most troublesome part to construct. It will be noted that it is necessary that the pump should not produce pulsations, as this would cause the level of the liquid in the manometer (*M*, fig. 1) to move up and down in time with the beats of the pump. It is essential that this should not happen, and a pump of the following design has been developed to overcome this particular difficulty. The air is pushed along a rubber tube by means of rollers. The details of the pump, which was made for me by Mr. Kelly, a mechanic attached to Trinity College, Dublin,

are as follows: The body of the pump consists of a brass tube, 1 (fig. 2 A), provided with a flange, 2. It is bolted to the rigid base plate, 3, by bolts passing through holes in this flange. The bottom of this tube is closed by the plug, 4, which screws in. A shaft, 5, is made to revolve inside this tube at about 20–40 r.p.m. A worm, 6, attached to the shaft of an electric motor meshes with the worm wheel, 7, attached to the shaft, 5. The motor is also securely bolted to the base plate. At the lower end the shaft bears in a hole in the plug, 4, but at the upper end ball-bearings are necessary.

Details of the construction of the "head" of the shaft appear in longitudinal section in fig. 2 A and in transverse section in figs. 2 B and 2 C. It consists of a solid brass cylinder provided with four longitudinal slots in which brass rollers, 8, fit loosely. These slots appear in longitudinal section in fig. 2 A and in transverse section in fig. 2 C. These rollers are pressed away from the core

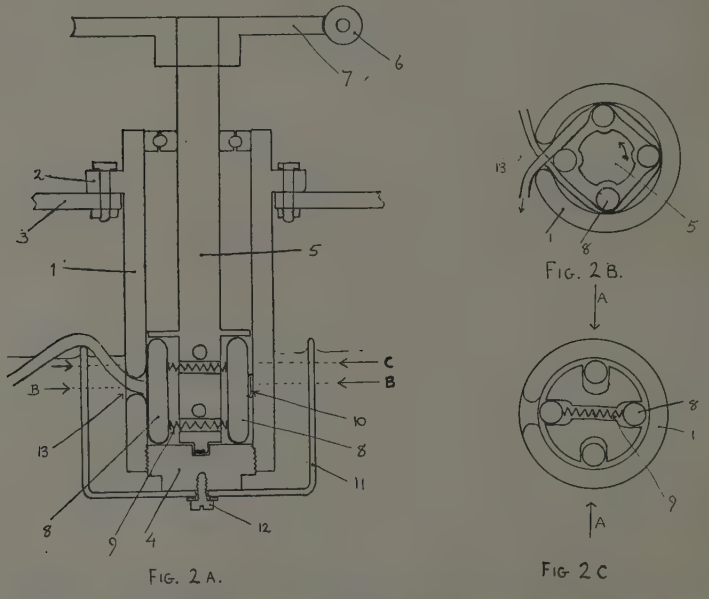

Fig. 2 A. Fig. 2 B. Fig. 2 C

Fig 2.—Diagrams illustrating construction of the gas-circulating pump. For description, see text.

of the shaft against the outer tube, 1, by light springs made from phosphor bronze wire (9). These springs pass through holes which are drilled horizontally through the shaft. As the diagrams show, one spring presses out the tops of a pair of rollers and another spring their lower ends. Fig. 2 B is a transverse section taken at the level indicated by the arrows labelled "B" in fig. 2 A. Fig. 2 C is a section taken at the level shown by the arrows labelled "C." Fig. 2 A is a longitudinal section taken through the plane of the slots.

The middle part of the "head" is turned down to a smaller diameter to leave clearance for the rubber tube, 10. This is threaded through a hole in the outer tube, 1, about 7 mm. in diameter, and is threaded round between the outer tube and the rollers. The springs (9) cause the rollers to press the rubber tube flat, as indicated in fig. 2 B. As the shaft revolves the rollers squeeze the air along the rubber tube in the direction of rotation of the shaft. The "intake" end of the rubber tube requires to be securely attached to a fixed part of the apparatus, otherwise the moving rollers drag the tube right through the pump. While the pump is working, this part of the tube, and also the parts of the rubber tube inside the brass cylinder are considerably stretched.

To prevent both undue stretching and also undue wear of the rubber, it is necessary to have the whole "head" of the pump immersed in a lubricating fluid. Accordingly a basin-shaped container, 11, is fixed by the bolt and washer, 12, to the base of the body of the pump. The only satisfactory lubricant was found to be glycerin. So the basin, 11, is filled with glycerin, and, as this is hygroscopic, it is necessary to renew it periodically, as the dilution with water destroys its lubricating properties. Fresh rubber tubing is readily fitted without dismantling the pump by inserting the end through the hole, 13, and turning the shaft round: the rollers drag the tube in, the output end is then pulled out through this hole, 13, and the intake end firmly fixed to some part of the apparatus. The rubber tube should be of the thin-walled type, and the strength of the springs so adjusted (by trial and error) that the rollers just, but only just, press the rubber tube flat. The pump is driven by a $\frac{1}{16}$ H.P. electric motor. The position of the pump is shown by *k* in fig. 1.

The plant chamber.—The form of this is a matter of indifference; a glass bottle fitted with a rubber bung, inlet, and outlet tubes has been used with success. This is shown diagrammatically as *j* in fig. 1. The bottle was painted black to darken the leaves.

The CO_2 absorbing tube.—The gas stream from the plant chamber bubbles through baryta, and in order that the formation of each bubble should be associated with as small a change of pressure as possible, this tube has to be of the "Pettenkofer" type. See fig. 1, *l*. The gas stream enters by the tube *e*. The three-way (T-type) tap *f* gives an alternative connection with the external atmosphere which is needed when it is required to fill the apparatus with nitrogen or a special gas mixture. For this purpose tap *f* must be placed in position 3, and tap *g* is also turned to position 3. When tap *g* is turned into position 4 the standard baryta runs out of the burette into the Pettenkofer tube, provided that tap *m* is in position 4, *i.e.*, in connection with the external atmosphere. The titration of the baryta is partly performed in situ, by running in standard HCl, containing phenol-phthalein, from the burette by opening tap *d* (tap *m* being in position 4). A stream of bubbles of air, freed from CO_2 by the soda-lime tube *h*, is drawn through by sucking from the opening *n*. This method of titration in situ has been copied from Blackman (1) (it is also employed by Wetzel (5)); but owing to the shape of the tube the mixing is incomplete, and the last few c.cs. of HCl are added to the mixture

outside the apparatus. For this purpose the tap *d* is turned in the opposite direction, and the contents of the Pettenkofer tube are blown out through the tube *b* into a beaker, and extra HCl is added from *a*. The neutralised liquid is used to wash out the Pettenkofer tube and the tube *e*, and is re-neutralised if necessary after this operation, and a second washing is carried out.

The washing is done by sucking the liquid back again into the tube from the beaker, by a mouth-piece attached to *n*. The baryta is not exposed to the air during titration until it is almost completely neutralised, and then only for a very short time, so that one obtains much of the advantage of the titrations in the enclosed CO_2-free atmosphere, which are the characteristic of Blackman's method (*loc. cit.*).

After refilling with a standard quantity of baryta, the taps are turned into the following positions:—tap *g* in position 3; tap *f* in position 4; tap *d*, off; tap *m* in position 3.

The manometer and oxygen generator.—This is fused onto the modified Pettenkofer tube. It is a three-limbed tube, the structure of which is made clear from fig. 1. A platinum wire attached inside to a spiral of copper wire, *r*, is fused into the middle limb. The left-hand limb is joined to the circulating gas system by the side tube, *m*. A piece of platinum foil is held in the middle of this limb by the tube, *o*, into the bottom of which it is fused. Electrical connection with this platinum electrode is made by means of the mercury which fills the tube, *o*. The three-limbed tube is filled so that the level of liquid is equal in the right and left limbs and just not touching the electrode, *p*: the middle limb is completely filled, and is used for the purpose of changing the liquid in the manometer periodically. This is made necessary by the gradual deposition of the copper from the solution onto the electrode. When tap *q* is open, the "spent" copper solution can be sucked out by a pipette, and fresh solution replaced, if both the compensator and the circulating system are placed in connection with the atmosphere.

As already described, this device secures the automatic addition of oxygen to the apparatus at the same rate as the plant removes it. The accuracy of its operation is obviously dependent on all parts of the apparatus being kept at exactly the same temperature, and vigorous stirring of the water-bath is therefore necessary.

Whilst actually in operation, the volume of the gas circulating over the respiring tissue goes through a characteristic cycle of changes which will be made clear by considering fig. 3. The original state of the system is indicated by fig. 3 a: the meniscus is just not touching the electrode, *p* (fig. 1). A very small decrease in the volume of the gas system causes the liquid to rise and just touch the electrode, so that the meniscus takes up the form shown in fig. 3 b.

Oxygen is now being added as the electrical circuit is completed, and the volume of the gas in the circulating stream increases. The meniscus moves to the position shown in fig. 3 c. The surface of the liquid is increased in area by this addition of oxygen, until finally the meniscus breaks away from the electrode

to the position shown in fig. 3 d. This breaks the circuit and addition of oxygen ceases. The system slowly returns to the position shown in fig. 3 a, as a result of the absorption of oxygen by the tissue, and the process is repeated. 8 volts is applied to the circuit, and with this voltage it is found that the time occupied to pass from stage "a" to stage "d" is under 5 minutes.

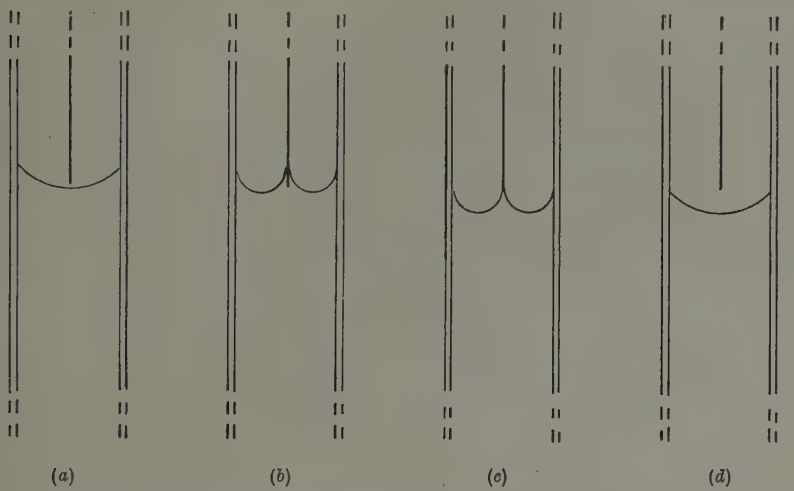

FIG. 3.—Positions of fluid in left-hand limb of the manometer—(*a*) just before touching electrode, (*b*) just after touching, (*c*) just before breaking of the circuit, (*d*) just after break of circuit.

The time occupied between stage "d" and stage "a" depends on the rate at which the tissue absorbs oxygen. The volume of oxygen added to the circulating gas stream during one complete cycle is found to be the same each time the cycle is completed (at a given constant temperature). It is clear that the difference in volume of the gas system in stage "a" and in stage "d" is determined by the diameter of the manometer tube, by the size and shape of the electrode, and by the surface tension of the liquid. This liquid changes slightly in composition during the course of its electrolysis, but the presence of copper hydroxide reduces the magnitude of this change in composition, and the resultant change in surface tension is apparently too slight to introduce serious error.

The volume of oxygen added during a given period of time is obtained by measuring with an ordinary gas burette the volume of oxygen + hydrogen given off from the electrodes (*u*, fig. 1) which are joined in series with the electrodes, *p* and *r*. This volume reduced to standard temperature and pressure is three times the volume (also at S.T.P.) of oxygen added into the apparatus at the electrode *p*.

The number of cycles is automatically recorded by means of an electromagnetic marker joined in series with the two sets of electrodes. The manner of its operation is made clear in the diagram, fig. 1. The electromagnet, *x*, moves the lever, *y*, which is pivoted at *z*, whenever actuated by the current flowing in the circuit *p-o-t-u-x-r*. The point of the lever writes on a drum rotated by a clockwork mechanism, so that whilst the liquid in the limb of the manometer is in the condition shown in fig. 3 *b* and *c*, the lever is raised. For 2–5 minutes during each cycle the lever is raised. During the rest of the "cycle" the lever is lowered and traces a horizontal line on the drum. Each time the oxygen is added the lever is raised for 2–5 minutes, and therefore makes a vertical mark on the drum at each "make" or "break" of contact.

The volume of oxygen added is the same each time an addition is made. In the case of the particular manometer tube and electrode used by the writer, this added volume is about 0·36 c.c. The distance between the consecutive vertical marks therefore gives the length of time required by the tissue to absorb 0·36 c.c. of oxygen. This automatic record of the oxygen additions is made by the apparatus during the night or during any enforced absence of the investigator. The frequency of the vertical marks made on the drum by the electromagnetic marker gives a direct measure of the fluctuating rate of oxygen intake during the night. The total number of marks gives the integrated quantity of oxygen added. This quantity is also obtained by making a reading of the gas evolved at *u* with the gas burette (*w*). An independent control of the automatic record is thus obtained. It must be remembered, of course, that the gas in the gas burette is at a different temperature and sometimes a different pressure from the gas in the apparatus.

To make the method clearer a typical graphical record is shown in fig. 1 of the following communication (this volume, p. 296) of the tracing made on the drum by the relay, and the curve showing the change in rate of oxygen intake with time which has been deduced from it. 20 grams of detached leaves of *Sedum praealtum* was the material used. It will be noted that at 7 p.m., when the leaves were placed in darkness, the duration of a single cycle was almost exactly 27 minutes. The rate of oxygen intake was therefore 0·36 c.c. per 27 minutes, which is 0·78 c.c. per hour. Shortly before midnight the duration of the complete cycle was only 16 minutes, so that the rate of oxygen intake was then 1·42 c.c. per hour. The "stepped" graphical record in fig. 1, *loc. cit.*, shows the rates of oxygen intake (in c.c. per hour) deduced from the record made on the drum.

Calibration and Testing of the Apparatus.

Two types of test experiment were carried out. In the first type, the Pettenkofer tube (*l*, fig. 1) was filled in the usual way with standard baryta, 25 c.c. of N/10 baryta was used. This is run into the Pettenkofer tube by turning tap *m* into position 4, and tap *f* into position 3. Then on turning tap *g* into position 4 from its normal position (position 3) the baryta runs in.

g is turned back again, and the taps *m* and *f* are turned to the normal positions (positions 3 and 4 respectively).

Some carbon dioxide was now introduced into the plant chamber, which in this series of experiments contained no plant material. This was done by turning tap *m* into position 4, and tap *f* into position 2: the tube *n* was then connected to a Kipp's apparatus generating carbon dioxide, and the pump was started and allowed to turn round about six to ten times. The taps were then turned into their normal positions and the tube *n* disconnected from the Kipp's apparatus. The level of the liquid in the left limb of the manometer was then adjusted so that it just did not touch the electrode *p*. The pump was then started. The air (containing added CO_2) thus circulated round the apparatus in the normal way. The CO_2 is, however, absorbed in the Pettenkofer tube, and there results a decrease in volume of the circulating gas stream, which is counteracted, as described, by the electrolytic addition of the same volume of oxygen from the electrode *p*.

The volume of carbon dioxide absorbed in the Pettenkofer tube ought obviously to be equal to the volume of oxygen liberated from *p*. The weight of carbon dioxide is obtained by titration of the baryta, as described. The volume of oxygen liberated at *p* is one-third of the volume of gas ($2H_2 + O_2$) liberated at *u*: this volume is obtained by using the gas burette (*w*). The total volume of gas in the circulating system will be the same as it was at the commencement of the experiment when the level of liquid in the left limb of the manometer (*M*) is just not touching the electrode *p*. The experiment is therefore stopped, and the titration of the baryta, and the reading of the gas burette made when the manometer liquid is in the position indicated above.

The results of three typical experiments of this type are given in the table appended below:—

TABLE 1.

Mg. CO_2 absorbed.	Vol. of CO_2 at 0° C. and 760 mm.	Vol. of O_2 at 0° C. and 760 mm.	$\frac{CO_2}{O_2}$
5·98	3·04	2·96	1·03
4·15	2·11	2·07	1·02
1·30	0·66	0·62	1·06

In the second and third columns respectively are given the volumes of the CO_2 absorbed and the volume of oxygen simultaneously introduced into the apparatus at standard pressure and temperature. The ratio of the volume of CO_2 to that of oxygen, which is given in the fifth column, should equal unity.

The slight deviations from unity actually shown are representative of the errors likely to be encountered when the apparatus is used to measure the oxygen intake and CO_2 output of plant tissues.

The second type of test experiment was carried out as follows: An experiment was started in the usual way, which was followed when the gas exchanges of living tissues were being investigated. The Pettenkofer tube was filled and

20 grams of young leaves of *Sedum praealtum* were put in the plant chamber. The liquid in the manometer was adjusted so as just not to touch the electrode *p*, and the taps put into their correct positions. The pump was then started.

Samples of gas were removed from the circulating system at various periods. The number of hours from the start of the experiment at which each sample was removed is indicated in the first column of Table 2. These samples were not obtained through the taps *g* or *m*, as the gas in the tubes attached to these taps is "stagnant." Instead, a third tube was fixed through the rubber bung of the plant chamber in addition to the inlet and outlet tubes. This was a capillary tube, and the gas sample was led out through it.

After the removal of the sample (about 5 c.c.), air was let into the apparatus to return the volume to its original value.

The sample was analysed with a capillary eudiometer apparatus of the type used by Bonnier and Mangin. The compositions of a number of samples of the circulating gas collected in this way are given in Table 2 below:—

TABLE 2.

Time from start of expt.	percent. oxygen.	percent. CO_2.
30 minutes	20·6	0·40
1 hour	20·2	0·69
5 hours	20·3	0·55
17 hours	20·3	0·53

It will be noted that the CO_2 content and also the oxygen content of the air in the plant chamber (and incidentally between the plant chamber and the Pettenkofer tube) do fluctuate to a slight extent. This change in composition is most marked during the first hour after the placing of the plants in the apparatus, and during subsequent periods a change of rate of carbon dioxide output by the tissue is accompanied by a slight change in composition of the air in the plant chamber. This source of error, which is inherent in the Pettenkofer method, has the effect of introducing a lag between the actual time of output of the carbon dioxide and the time at which the gas is absorbed and estimated.

This error is greatest at the beginning of the experiment.

It will be noted from Table 2 that, after the first hour in the case of the experiment recorded, the plant chamber contained 0·69 % of CO_2: the volume of air in the chamber and attached tube not occupied by the tissue was about 30 c.c., and contained, therefore, 0·21 c.c. of carbon dioxide. Since the actual measured rate of output of carbon dioxide in the case of this particular sample of leaves during the first three hours after darkening was 3·6 c.c. per hour, the true rate should be 3·81 c.c. per hour.

The apparent CO_2 output during the first hour is, therefore, some 6 % too low. During the next two hours the change in composition of the gas in the plant chamber is negligible, and it follows that the measured and actual carbon dioxide outputs are about equal.

Corresponding errors are introduced into the records of the oxygen intake by this lag in carbon dioxide absorption. Thus, in the first hour in the experiment recorded above the oxygen intake was recorded as 2·0 c.c. per hour, instead of approximately 2·21 c.c.

The apparent respiratory quotient CO_2/O_2 is in this case, during the first hour of starvation of the leaves, 1·80; this value is somewhat too high on account of the lag due the apparatus, and the analyses recorded above indicate that the real value should be 1·72.

This error only attains such large dimensions when the rate of carbon dioxide output changes rapidly. This is, of course, most marked when the plants are first put into the apparatus, the rate of CO_2 formation in the apparatus rapidly rising from zero (before addition of the living tissue) to a value of say 3–4 c.c. per hour. The error would be greatly reduced by having a gas circulating pump which circulated the gas mixture more rapidly. In the apparatus described the gas is circulated at the rate of about 500 to 600 c.c. per hour.

Conclusion.

In concluding this account, it should be pointed out that this apparatus is designed not to determine the volume of oxygen absorbed in unit time, but the time taken by the plant to absorb "unit" volume of oxygen. The effective unit is, for this apparatus, 0·36 c.c. These times are recorded graphically on a moving drum. The apparatus is independent of changes in atmospheric pressure, as it operates on the principle of a differential manometer.

The composition of the air surrounding the plant remains approximately constant, as oxygen is supplied by an automatic device to the tissue at the same rate as it is consumed.

Greater constancy would be attained using a gas circulating pump having a greater output.

The carbon dioxide simultaneously evolved from the tissue is absorbed in a tube of the "Pettenkofer" type, the contents of which can be estimated by titration in situ.

Literature.

1. BLACKMAN, F. F.—Experimental Researches on Vegetable Assimilation and Respiration, I, Phil. Trans. Roy. Soc., B, 186, 503, 1895.
2. FERNANDES, D. S.—Aerobe und Anaerobe Atmung bei Keimlingen von *Pisum sativum*. Rec. Trav. Bot. Néer., **20**, 107, 1923.
3. GODWIN, H.—Ph.D. Thesis. Cambridge University, 1925.
4. THODAY, D.—On the Capillary Eudiometer Apparatus of Bonnier and Mangin, etc. Ann. Bot. **27**, 565, 1913.
5. WETZEL, K.—Zur Physiologie der organischen Säuren in grünen Pflanzen, IV. Planta **4**, 476, 1927.

No. 24.

THE RESPIRATORY QUOTIENTS OF SUCCULENT PLANTS.

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[Communicated by Professor H. H. Dixon.]

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Since the time of De Saussure it has been known that the respiratory quotient (the ratio volume of CO_2 evolved/volume of O_2 absorbed) differs very markedly from unity in the case of the succulent plants, though having the value of unity most commonly in ordinary thin-leaved plants.

The earlier investigators have established in the cases of the leaves of certain members of the Crassulaceae and the joints of Opuntia that during the period of a few hours after the darkening of the organ the ratio of CO_2/O_2 is considered smaller than unity. Values lower than unity and as low as 0·016 have been recorded by such investigators as Aubert (2), Astruc (1), and Richards (6). These low values (surprisingly low in some cases) are explained, no doubt correctly, as being caused by the large accumulation of malic acid in the tissues, at the expense of carbohydrate. Thus a considerable absorption of oxygen occurs which is associated with no evolution of carbon dioxide.

In all cases these investigators have contented themselves with measuring the R.Q. during the first few hours after the plant tissue was darkened. Only one measurement is in general recorded. The changes in the value of the R.Q. which take place in the darkened leaf during the accumulation of acid have not been investigated at all adequately. It has, however, been shown by Astruc that, if a leaf of Sempervivum is left in darkness during the night, and is then placed in darkness in the apparatus used for the measurement of its gaseous exchanges, the R.Q. (CO_2/O_2) has a value above unity. This is ascribed by him to the fact that under these conditions the malic acid accumulated during the night disappears.

The behaviour of the leaves may be summarised briefly by stating that when darkened the acid-content rises until the maximum value is attained about eight hours after the leaves were darkened. During the following 8 to 12 hours the acid-content decreases. Still later a further slight rise, followed by a slow and long-continued decrease in acidity, takes place.

Thus it is to be expected that this constantly changing acid-content would have its counterpart in a constantly changing value of the respiratory quotient. This is shown to be actually the case in this preliminary note.

Astruc measured the values of the R.Qs. at the two stages noted above; even he, however, made no attempt to obtain a continuous record of the changing values. Some of his results are of very great interest.

The disappearance of malic acid in continued darkness has been supposed by previous investigators to be due to its conversion into carbon dioxide; it is supposed by almost all investigators that the malic acid forms the substrate from which CO_2 is evolved. Its complete oxidation to carbon dioxide and water in respiration would cause the R.Q. to have the value of 1·33:

$$C_4H_6O_5 + 3O_2 = 4CO_2 + 3H_2O.$$

Incomplete oxidation of the acid might cause the R.Q. to have a lower value, but it is significant that the highest possible value is 1·33. It should be noted that no doubt exists that the disappearing acid is malic acid.

The values recorded by Astruc (*loc. cit.*) are in many cases 1·33 or lower, but it is significant that in many cases the R.Qs. were very much higher; such values as 1·50, 1·48, 1·53, 1·75, 1·78, 2·41, and 1·42 were also obtained. The significance of these high values has not been discussed adequately as yet.

In general it has been assumed that a high initial value of the R.Q. is due to the circumstance that a relatively cool leaf placed in a warm respiration chamber evolves carbon dioxide both by respiration and by the gas passing out of solution in the tissue owing to its lower solubility at the higher temperature.

It is, however, extremely doubtful if this is the case (Blackman, 4), as the lower solubility is compensated for by the fact that at higher temperatures the partial pressure of CO_2 in the intercellular spaces is raised, and this forces more CO_2 into solution in the tissue fluids.

The present work embodies some results in which values of the R.Q. as high as, and in certain cases higher than, those recorded by Astruc are found. These were obtained under conditions in which the above criticism, that some of the CO_2 has come out of solution (even if it were valid), does not apply. It seems that these high values must be accepted as the actual respiratory ratios, and that they are not brought about by physical effects connected with the solubilities of the gases.

These values of the R.Q. above the maximum value for the simple oxidation of malic acid, which is 1·33, can only be brought about by the existence of some source of supply of oxygen other than atmospheric oxygen. Similar high values for the ratio CO_2/O_2 are commonly found among the yeasts and mould fungi, where they are in general supposed to be due to the formation of reduced compounds like alcohol from carbohydrate. In this case oxygen may be regarded as being made available by its transference from some of the products of glycolysis (which are thereby reduced) to others, which become

oxidised and may yield carbon dioxide, even in the complete absence of atmospheric oxygen.

It is suggested here that the high R.Qs. found whilst the malic acid is disappearing from the leaves of the Crassulaceae in darkness are due to the presence of an oxygen supply of a similar nature. In this case, however, reduced compounds of the nature of alcohol are not found in the leaves. On the other hand, a source of oxygen exists in the malic acid which would become available for oxidations if the malic acid were reduced. The conversion of malic acid into carbohydrate, for example, would render oxygen available as follows:—

$$3C_4H_6O_5 + 3H_2O = 2C_6H_{12}O_6 + 6O.$$

If half of the carbohydrate formed were then completely oxidised to CO_2 and water and half stored in the tissues, evidently, for every 3 molecules of malic acid disappearing, 6 of CO_2 would be evolved, and only 3 molecules of oxygen would be taken from the air: the R.Q. would, in this case, be 2·0, and it will be noted that the quantity of carbon disappearing from the form of malic acid is double the quantity which is evolved as CO_2.

There is direct evidence (3) that conditions are frequently realised when it is found that the quantity of malic acid carbon disappearing from darkened leaves (severed from the plant) is considerably in excess of the quantity of carbon evolved as carbon dioxide. In the case of *Sedum praealtum*, which yielded the material used in the present preliminary investigation, this rapid disappearance of malic acid is found most conspicuously when leaves which have been kept at 1°–3° C. for two or three days are suddenly warmed to, say, 25° C. The cool leaves are rich in malic acid, and this acid disappears when the leaves are warmed: at the same time a large excess output of carbon dioxide occurs, but it is significant that the quantity of carbon evolved is about one-third of the quantity which disappears from the form of malic acid in the tissue.

It consequently seems almost certain that the disappearing malic acid is converted into some neutral compound which accumulates in the tissues. Oxidation products of malic acid are all acids, and one is therefore led to infer that the disappearance of acid is due to its reduction. Reduction of the malic acid, as pointed out above, renders oxygen available for other oxidations, and in consequence of this a considerable carbon dioxide output is associated with a small intake of oxygen from the air. The R.Q. exceeds unity by an amount dependent on the ratio of malic acid carbon lost to carbon dioxide carbon formed.

Assuming, as before, that the malic acid is converted into either carbohydrate or, at least, some compound of the composition $C_nH_{2n}O_n$, it follows, as pointed out already, that when the ratio of malic acid carbon lost/carbon dioxide carbon formed is 2, the ratio CO_2/O_2 is also 2. With malic acid carbon/carbon dioxide carbon ratios of the values 0, 1, 2, 3, and 4, the corresponding values for the R.Q. would be 1·0, 1·33, 2·0, 4·0, and infinity, respectively.

This preliminary note is concerned solely with the presentation of some experiments confirming and amplifying Astruc's results, pointing out, moreover, that the only inference possible from them is that the malic acid is caused to disappear by reduction, and not by oxidation into CO_2 and water. This summer the attempt will be made to correlate the values of the R.Q. with the corresponding values of the malic acid loss/CO_2 ratios.

Experimental Results.

I. Leaves of *Sedum praealtum* were collected, 20 grams were weighed out and placed in darkness at 7 p.m. on 3rd July in the plant chamber of the apparatus described in the preceding paper.

A "continuous" record of the rate of oxygen intake was obtained by the method described. The quantities of carbon dioxide were estimated approximately every two hours; the actual times at which the estimations were made are indicated in the graphical record (fig. 1).

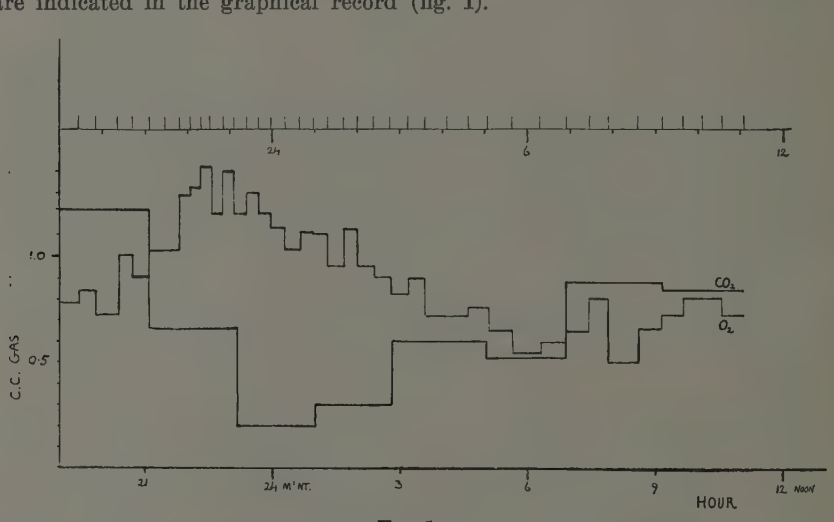

FIG. 1.

The rate of carbon dioxide output is expressed as the volume of moist CO_2 at 755 mm. pressure and 24° C. evolved per hour per 20 grams fresh weight of leaves. These arbitrary units are given in this graph, since the rates of oxygen intake are also given in c.c. per hour per 20 grams fresh weight at the same temperature and pressure. The volumes of oxygen as given by the automatic record are the volumes at the temperature of the apparatus, 24° C., and at the pressure at which the air was enclosed in the apparatus, in this case 755 mm. These volumes of carbon dioxide output and oxygen intake should be multiplied by 0·90 to convert them to volumes at N.T.P. In the

other graphical records the rates of gas exchange are expressed in the normal way as c.c. per hour at N.T.P. per 100 grams fresh weight.

It will be noted that the rate of CO_2 output falls from its initial high value to a minimum value attained during the third two-hour period. The rate then rises, and about 18 hours after the starvation period commenced it attains an equilibrium value which is maintained unchanged for some days. This equilibrum rate is about 3·8 c.c. at N.T.P. per 100 grams fresh weight per hour. The initial rate of oxygen intake is considerably lower than that of the CO_2 output ($CO_2/O_2 = 1·56$), but the rate rapidly rises to a maximum value attained about 3 or 4 hours after darkening. The rate then falls to a minimum value, attained about 11 hours after darkening, and rises, finally, somewhat erratically to a value which approaches that of the carbon dioxide output.

The average values of the R.Q. during each two-hour period are as follows: 1st, 1·46; 2nd, 0·55; 3rd, 0·17; 4th, 0·30; 5th, 0·78; 6th, 0·86; 7th, 1·40; 8th, 1·11. The low values obtaining between the 2nd and 12th hours are connected with the accumulation of malic acid at the expense of carbohydrate. These values will not be further discussed at present.

After the 12th hour the R.Q. rises above unity, actually attaining a value of 1·4 in this case.

Since the leaves had been kept at a constant temperature for the previous 12 hours, this high ratio cannot be explained as due to a physical effect of gas coming out of solution (after the manner of Aubert (2) and others). It can only be due to the actual production of CO_2 and utilisation of oxygen in the metabolism of the leaves.

Actually, a source of error exists, as was pointed out after the description of the apparatus, in the fact that an increase in the rate of CO_2 production by the leaves is associated with a slight increase in the concentration of CO_2 in the apparatus. The effect of this is to make the experimentally determined R.Q. appear slightly more divergent from unity than the real value. The magnitude of this error can be roughly evaluated, as follows:—During the 6th two-hour period the rate of CO_2 output was 0·6 c.c. per hour, and the velocity of the gas stream about 600 c.c. per hour; the circulating gas contained therefore 0·1 % CO_2. During the next two-hour period the data similarly show that the gas contained 0·15 % CO_2. The volume of the space containing this gas mixture is about 30 c.c.; this space, therefore, contains 0·015 c.c. more carbon dioxide during the seventh two-hour period than during the sixth, and, similarly, 0·015 c.c. less oxygen. The ratio CO_2/O_2, which appears to be 0·88/0·63 (= 1·40), should really be $(0·88 + 0·015)/(0·63 + 0·015)$, which equals 1·39.

In this case the ratio is not very strikingly higher than 1·33, the maximum which could be attained if malic acid becomes oxidised in the tissues. It is, however, significantly higher.

II. An essentially similar experiment was started on July 6 at 3.45 p.m.; 20 grams of detached leaves being again used. The temperature was again 24° C. during the experiment. In the graphical record, fig. 2, the rates of gas exchange are expressed as numbers of c.cs. at N.T.P. per hour per 100 grams fresh weight.

The significance of certain features of the record has been discussed in connection with some earlier work (3), and need not be dealt with here again. Many differences of detail between the quantitative results in experiments I and II have been shown to be connected with the hour of day at which the leaves were darkened.

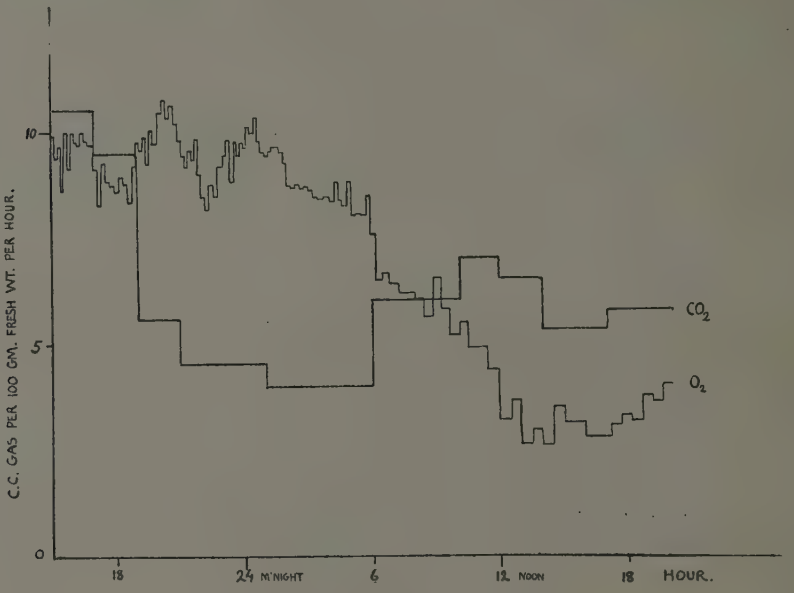

FIG. 2.

What is of special interest in this connection is that the R.Q. remains above 1·33 for some time, and that the maximum value is 2·15. After making allowance for the error due to the lag of the apparatus, the value of this ratio could not be under 2·05.

The conclusion is, therefore, inevitable that the true respiratory ratio considerably exceeds the maximum value theoretically possible for the oxidation of malic acid at the stage of starvation at which this acid is disappearing most rapidly.

Conclusion.

When excised leaves of *Sedum praealtum* are darkened, the rates of CO_2 output and oxygen intake undergo a series of characteristic changes. The rate

of CO_2 output falls to a minimum value attained about the 6th hour after darkening, and then rises to a value which remains nearly constant for many days.

At the same time the rate of oxygen intake at first rises to a maximum value and then falls to a minimum value, finally rising so that the R.Q. eventually attains the value of unity.

Thus the R.Q. is considerably lower than unity during the phase of starvation in which carbohydrate is being converted into malic acid.

It greatly exceeds unity in the subsequent phase, during which the acid formed during the first phase disappears. Maximum values for the R.Q. of 1·39 and 2·05 are recorded here, and it is pointed out that somewhat similar values have been recorded previously.

It should be mentioned that other experiments yielding similar results have been made which are not recorded here.

It is shown that these high values are inconsistent with the view that malic acid disappearance is due to its oxidation.

These data therefore provide additional evidence in favour of the view advocated before (3) that malic acid forms a link in a carbon cycle in succulent plants similar in some respects to the carbon cycle of apple deduced by Blackman (5).

The sequence of changes postulated is of the type

$$\begin{array}{l} \text{carbohydrate} \longleftarrow \text{polysaccharide,} \\ \quad\downarrow \\ \text{(intermediates)} \longrightarrow \text{malic acid.} \\ \quad\downarrow \\ \quad CO_2 \end{array}$$

My best thanks are due to Professor Dixon, who kindly provided me with most of the apparatus used, and with all the facilities of his laboratory in which the work leading up to this communication was done.

Literature.

1. ASTRUC, A.—Recherches sur l'Acidité Végétale. Ann. Sci. Nat. Bot., Sér. 8, Vol **27**, 1, 1903.
2. AUBERT, E.—Recherches sur la Respiration et l 'Assimilation des Plantes Grasses. Rev. Gen. Bot., **4**, 203, 1892.
3. BENNET-CLARK, T. A. — Ph.D. Thesis, Cambridge University, 1930, and Report Internl. Bot. Congress, 1930, p. 425.
4. BLACKMAN, F. F., and PARIJA, P.—Analytic Studies in Plant Respiration, I. Proc. Roy. Soc., B, **103**, 412, 1928.
5. BLACKMAN, F. F.—Analytic Studies in Plant Respiration, III. Proc. Roy. Soc., B, **103**, 491, 1928.
6. RICHARDS, H. M.—Acidity and Gas Interchange in Cacti. Publ. No. 209; Carnegie Inst., Washington, 1915.

No. 25.

ON THE CULTIVATION IN ARTIFICIAL MEDIA OF *CATENARIA ANGUILLULAE*, A CHYTRIDIACEAN PARASITE OF THE OVA OF THE LIVER FLUKE, *FASCIOLA HEPATICA*.

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AND

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(Plates 13–18.)

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Introduction.

In this paper an account is given of the growth on artificial media of the Chytridiacean fungus, *Catenaria anguillulae* (Sorokin). In a previous communication from this laboratory [Bayley Butler and Buckley, 1927], the occurrence of *C. anguillulae* as a parasite in the eggs of *Fasciola hepatica* was described, and an account of its life-history in this host was given. E. J. Butler (1928) described in detail the morphology of the fungus as it occurred in samples of infected fluke eggs sent to him, and discussed the relations of this example of *C. anguillulae* with some of those hitherto recorded. The previous records referred to by him were those of Sorokin (1876), Dangeard (1885, 1886), and Constantineanu (1901), together with the debatable records described as *Chytridium zootocum* by Braun, A. (1856), and as "A fresh water alga," by Villot, A. (1874). He also discussed the doubtful species *C. pygmaea* of J. L. Serbinov (1907).

J. J. C. Buckley and P. A. Clapham (1929) obtained an infection of *C. anguillulae* on several occasions from laboratory tap water in London. They found in their laboratory water-supply tanks a sediment which contained Infusoria, Rotifers, and other forms likely to act as hosts for the fungus. They exposed uninfected eggs of the Cestode, *Dibothriocephalus latus*, to this sediment in a petri dish. The eggs developed an infection of Catenaria, thus

confirming the suggestion put forward in 1927, that the original infection of liver fluke eggs, which occurred in this laboratory, originated from the Dublin City Water Supply. Buckley and Clapham also recorded the parasitism by *C. anguillulae* of the eggs of the Helminths, *Dibothriocephalus Mansoni*, and *Parascaris equorum*, together with that of the mite *Rhizoglyphus echinopus*. They describe and figure the occurrence of resting spores of *C. anguillulae* in the eggs of *D. latus* and *Fasciola hepatica*. Another record of *C. anguillulae* is that of Maupas (*vide* Seurat, L.G., 1920) in several species of free living Nematodes, as well as on the males of *Haemonchus contortus*, and the larvae of *Chabertia ovina*.

Buckley and Clapham (1929) sterilised *F. hepatica* eggs, and subjected them to infection from *C. anguillulae* growing in *D. latus* eggs. They found that after five days 55 %, and after eight days 62·1 %, were infected in tap water as a medium. They used from 3,000 to 4,000 ova in each culture. It is evident, therefore, that, under laboratory conditions, a very high percentage of infection of the eggs of the liver fluke by *C. anguillulae* takes place, even at normal temperatures, and within a short period.

In the earlier communication from this laboratory, a high rate of infection of living eggs was reported, and it was found that no infected egg proceeded with its development. This observation has been confirmed in the course of the present investigation. The possibility of using *C. anguillulae* as a means of checking the infection of snails by miracidia was suggested, and an attempt to culture the fungus on artificial media was proposed. The present paper deals with the results of experiments with this object in view.

Buckley and Clapham carried out a series of experiments with a view to determining the possibility of the utilisation of *C. anguillulae* for destroying Helminth eggs in sewage. The problem is of considerable importance in areas of endemic helminthiasis, since Helminth ova will pass through septic tank treatment without injury. They found that, while *C. anguillulae* will live and reproduce in water containing septic substances, the development of the fungus is inhibited to a marked degree, the cultures in tap water having a higher percentage of infection. They also found that *C. anguillulae* can develop under anaerobic or nearly anaerobic conditions, and that the rate of development is not slowed down. A short period of desiccation was found not to injure Catenaria cultures seriously, 91 % sporing after 12 hours, but only 4 % spored after 120 hours desiccation. Attempts made to culture the fungus in putrefactive and semifluid conditions were disappointing, and it would appear that *C. anguillulae* flourishes best in comparatively fresh conditions.

These observations, though not favourable to the utilisation of the fungus in the destruction of Helminth eggs in sewage, render it more hopeful as a possible biological control of liver-fluke disease. It was found that optimum infection occurred in ordinary fresh tap water, conditions similar to those obtaining in the ponds and streams in which infection of the snails by fluke miracidia occurs.

Host range.

If the fungus were confined to Helminth eggs, as hosts, the prospects of maintaining an infection, subsequent to the distribution of an artificial culture in fluke-infested marshy land, would depend on the presence of a continuous supply of Helminth eggs. Fortunately Catenaria is not so confined, as it has been recorded as infecting the following hosts also:—

1. Eel worms, species not stated, Sorokin (1876). The Eel worms in culture were eventually killed off by an epidemic of fungus diseases due to several different Chytridiaceae and Ancylistaceae, including *C. anguillulae.*

2. Encysted Infusoria. Sorokin (1876).

3. Eggs of various aquatic "animalculae," including possibly rotifer eggs. Sorokin (1876).

4. Several species of hair-worms of the genus Gordius. Villot, A. (1874).

5. *Nitella tenuissima.* Dangeard (1886).

6. *Haemonchus contortus,* male. Maupus (*vide* Seurat, 1920).

7. *Chabertia ovina,* larvae. Maupas (*vide* Seurat, 1920).

8. Eggs of *Rhizoglyphus echinopus.* (Buckley and Clapham, 1929.)

In view of the varied character of these hosts, which belong to widely separated phyla, and include both plant and animal organisms, it is not unreasonable to suppose that other hosts than those already recorded may serve to maintain an infection under field conditions. Experiments are in progress to determine what other common fresh water organisms are susceptible to parasitism by *C. anguillulae.*

C. H. Barlow (1925), who studied the life history of *Fasciolopsis buski,* the human liver-fluke, in China, states: "Freezing kills many eggs, but the greatest number is killed by natural enemies. These consist of diatoms, algae, which look like Ankistrodesmus and Protosiphon, and various ciliated Protozoa." As previously mentioned, the so-called "algae" figured are almost certainly a species of Catenaria, if not *C. anguillulae.*

Natural Enemies of Catenaria anguillulae.

With a view to determining the possible persistence of a culture under field conditions, observations are being made to determine what natural enemies prey on *C. anguillulae,* or its zoospores. A Heliozoon, *Actinophrys* (sp.), was observed catching and devouring zoospores (see fig. 1, Pl. 13.) Approximately twelve were seen to be captured by the radiating pseudopodia. Clinton (1902) observed in relation to *Cladochytrium alismatis,* "Despite the luxuriant production of zoospores, there are a number of causes that may prevent their forming sporangia. In the first place, they seem to be, in their fresh condition, a coveted prey for a number of lower animals. In cell cultures amœbae were

seen that had devoured eight or more. Even some of the Heliozoa, scarcely larger than a zoospore, were seen to catch as many as six before they became apparently satisfied. But the most ravenous enemies were occasional Rotifers which did not seem satisfied with several hundred."

Atkinson (1909) also records the attack by *Actinophrys* (sp.) on the zoospores of *Lagenidium Rabenhorstii.*

Culture on Artificial Media.

No difficulty is experienced in maintaining the cultures on fluke ova at ordinary temperatures in the laboratory; the culture originally found in 1926 having been maintained up to the present by merely renewing the supplies of fluke ova at intervals. The culture on fluke ova is so easily maintained in a laboratory, that *C. anguillulae* would form a very convenient type for class demonstration. A supply of fluke ova once obtained can be kept in plugged tubes of water for prolonged periods if sterilised.

With a view to determining the possibility of culturing the fungus in bulk on artificial media, and ascertaining the conditions necessary for its growth, experiments were carried out with hanging drop preparations. Unfortunately, there was little help to be obtained from existing literature as to methods of procedure. When this investigation was first undertaken, no record of the cultivation on an artificial medium of an undoubted parasitic Chytrid could be traced, though, owing to the scattered nature of the literature, it is quite possible that some reference may have been overlooked. Constant allusions to "cultures" occur, but these usually relate to the growth in a laboratory of a fungus on its host organism, or the germination of spores in the vicinity of the host. In the case of saprophytes, growth on their normal nutrient substratum is also referred to as a "culture."

The most exhaustive attempt to culture any of these fungi was undertaken by Ashworth (1922). He describes attempts to cultivate in vitro, *Rhinosporidium seeberi* (Wernick, 1903). This organism was formerly referred to the Sporozoa and placed in the Haplosporidia, but Ashworth, who made a detailed cytological study of the species, considers that Rhinosporidium should be included in the Chytridineae, and provisionally placed near the Olpidiaceae. Ramsbottom (1931) follows Ashworth by including his account of Rhinosporidium under the Chytridiales.

Ashworth refers to attempts by Tirumurti (1914) to cultivate the organism in hydrocele fluid, by Dr. Rettie in human serum, ox serum, horse serum, agar and serum, bread agar, Sabouraud's medium, and also in hanging drop preparations with human serum, normal saline, mammalian Ringer, rain water, bread agar with fruit juices, and Sabouraud's medium, with or without infusion of cabbage and carrot. The cultures were kept, some at room temperature and others at 23° C., but in none of them has any growth been found to take place. He also refers to attempts by Dr. Malcolm Wilson to germinate the spores of Rhinosporidium. Wilson placed them in hanging drops of tap water,

bouillon, prune extract, 2 % solution of glucose in water, and Uschinsky's solution. He also sowed spores on bouillon agar. He reports that in all cases the results were negative. Dr. Rettie placed spores from sporangia just discharged by a patient, on the surface of Sabouraud's medium contained in an Erlenmeyer flask. This was kept under observation for two or three weeks, and it seemed, like the culture tubes and hanging drops, to be unproductive. Examination some five weeks later showed that two small colonies of organisms had developed. These colonies consisted of bacteria, together with numerous specimens of an organism containing a fairly large karyosome. As the culture proved not to be pure, these cells may have developed from one or more organisms adherent to the surface of the sporangia, but there remains the possibility that they have arisen from the spores of Rhinosporidium.

Apart from the admittedly doubtful culture of Rhinosporidium referred to by Ashworth, the only other record of artificial culture we were able to trace was that of *Coccidioides immitis* (Stiles), a fungus whose systematic position is a cause of controversy. Da Fonseca (1928) refers Rhinosporidium and Coccidioides to the Protomycetaceae, also a family of doubtful and isolated systematic position, usually considered as closely allied to the Ascomycetes. Ramsbottom (1931) considers that the internal spores and general structure suggest that the proper position of Coccidioides is near Rhinosporidium, though he states that the occurrence of mycelium in cultures is puzzling. He includes it and Rhinosporidium under the heading "Chytridiales." The fungus has been cultivated anaerobically by MacNeal and Taylor (1914). When grown on culture media the cysts give rise to septate mycelial filaments. Arthrospores are produced abundantly by the mycelium. Older colonies form aerial hyphae with Chlamydospores isolated or in chains.

Since this investigation was undertaken, Karling (1928) recorded the germination of zoospores of the saprophytic Chytrid, *Entophlyctis heliomorpha*, in a drop of water in which a bit of host tissue had been macerated. *E. heliomorpha* occurs in the dead nodal, internodal, and corticating cells of the Characeae. He describes and figures the germination of a zoospore and the growth in culture of the thallus for 24 hours. By this time a young sporangium had been formed, which showed the commencement of a dehiscence tube, as well as four rhizoids. Apparently the growth ceased at this point and the sporangium did not proceed to dehiscence. Karling states that the growth of the germ tube produced by the spore in his preparations was unusually long, and considerable time was occupied in its growth.

Sparrow (1931) recorded the cultivation of *Cladochytrium Nowakowskii* n. sp. in an artificial medium. This species is parasitic on *Spirogyra crassa* (Kutz), two undetermined species of Oedogonium, young thalli of Coleochaete, and several other members of the Chlorophyceae. In a foot-note after his paper had gone to press, Sparrow mentioned that he had had an opportunity of examining the MSS. of Dr. Karling's paper on *C. replicatum* n. sp. (Karling, 1931). He considers that it is more than probable that both his new species

and Karling's *C. replicatum* are identical with *C. polystomum* (Zopf). Sparrow states, "The fungus was cultured for a time on bactocornmeal agar. It seemed to have no difficulty in maintaining an existence on this medium, but, due to the slow rate of growth, it was impossible to free it from bacteria. The mycelium, in culture, was characterised by the formation of an abundance of septate turbinate cells." Sparrow does not state whether the fungus produced sporangia in the medium, or if the zoospores germinated.

Description of Culture Methods adopted.

The procedure adopted was as follows:—The required number of infected ova were first isolated by means of sterile needles on a flamed cover glass. From this they were transferred to another sterile cover glass, and a drop of the medium added. One of the first difficulties that had to be overcome was that the ova are often encrusted with epiphytes, Algal and Fungoid. When such ova are introduced into the experimental medium, these adventitious organisms flourish exceedingly, obscuring the ova and ousting the Catenaria, which it is desired to cultivate. Various measures were resorted to in an endeavour to obviate this trouble and procure clean ova. The first method attempted was to shake the ova vigorously for twenty minutes in sterilized water, and allow them to settle, decanting the supernatant water as soon as settlement had occurred. On account of their larger size, it was hoped the ova would sediment more rapidly than the epiphytic growths, which the shaking had been intended to detach. The process was repeated several times in successive washes. This method, however, failed to reduce to any appreciable extent the epiphytic organisms. Later, it was found that by using boiled ova, and renewing the cultures frequently, the adventitious organisms could be held in check, and embarrassment in microscopic observation avoided. It was found that if the cultures were kept in the dark, these growths were considerably reduced, though the growth of Catenaria was not affected.

Material for Media.

Agar-agar 0·25 % *in water* was found to give a jelly of the required consistency.

Extract of Liver Fluke Ova. The ova were obtained by expression from the bile duct and from the gall bladder, and cleaned by repeated sedimentation in water. The washing removed bile, blood, mucus, and other debris, and the sediment consisted of practically pure fluke ova.

Various methods of extracting the contents of the ova were tried. Simple grinding in a pestle and mortar was unsatisfactory, as the majority of the ova remained intact after prolonged grinding. Two methods were adopted, both of which gave positive results.

Extract of Fluke Ova (*A*) was obtained by crushing 2 c.c. of ova, a small quantity at a time, between glass slides, and rinsing the slides in 4 c.c. of

water. The extract thus obtained was filtered and boiled (to keep down infection).

Extract of Fluke Ova (*B*).—2 c.c. of ova were dried in a desiccator over calcium chloride. The dry ova were ground with kieselguhr in an agate pestle and mortar until a microscopic examination showed that practically all were crushed into fragments. The ground mixture was added to 2 c.c. of water, well shaken and centrifuged. The supernatant liquid formed extract B, which was boiled, and kept in a tube plugged with cotton wool. The extract retained its efficiency for several weeks, in spite of repeated boiling. The extract can be dried and redissolved without injury.

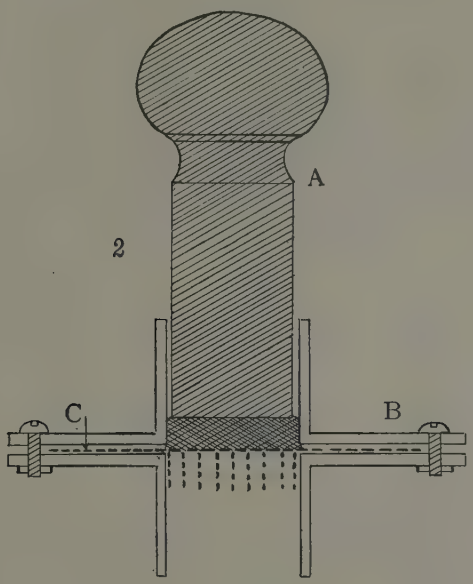

Fig. 2.

Coagulated Egg-Albumen Flakes.—We are indebted to Dr. E. J. Butler for the suggestion that zoospores might need a solid nidus in which to develop. He suggested experimenting with egg albumen. In order to obtain fine flakes of coagulated albumen, which could be observed under the microscope, a miniature press was used (Fig. 2). Two flanged brass sockets, such as are used for curtain rods, having a bore of 1″, were bolted together, holding a fine meshed copper gauze (50 meshes to 1″) between them. The entire apparatus was silver-plated. Egg albumen, either alone or mixed with fluke ova extract, was placed in the upper socket, and coagulated in a water bath. A box-wood piston forced the coagulated albumen through the gauze into the lower socket, forming fine shreds, which, when shaken in water, broke up into short lengths, forming

in fact a kind of artificial ovum. The suspension of flakes was autoclaved, and added to the medium.

Extract of Fluke.—As flukes can be obtained in greater bulk than fluke ova, the possibility of using entire fluke as a substitute for fluke ova suggested itself. The extract of fluke was made by washing and desiccating fresh flukes and grinding up with kieselguhr, as in extract of fluke ova B.

Various combinations of these media were tested. Negative results were obtained, except with the following:—

1. Agar, and fluke ova extract A in equal quantities.

2. Agar, fluke ova extract A, and flaked coagulated egg albumen in equal quantities.

3. Fluke ova extract A, and flaked coagulated egg albumen in equal quantities.

4. Agar and fluke ova extract B in equal quantities.

As only small quantities of fluke ova extract were available, the pH was determined by the capillator method, using Bromothymol Blue as indicator.

The results were as follows:—

	pH
Laboratory tap water	6·9
Extract of fluke ova A in laboratory tap water	6·9
Extract of liver fluke in laboratory tap water	6·2
0·25 per cent. agar-agar in laboratory tap water ...	6·9
Extract of fluke ova B in laboratory tap water	6·5

Description of First Outgrowth of Catenaria.

A hanging-drop preparation was set up on January 25th, 1928, using agar-agar and extract of fluke ova A in equal proportions. On January 27th one of the infected ova, which contained seven internal sporangia, was observed to have a detached operculum. Four of the internal sporangia had formed visible dehiscence tubes. The dehiscence tubes of the other sporangia, though not visible, probably opened on the underside of the ovum. One sporangium (X, Figs. 3 and 4, Pl. 13), lying near the opercular aperture, seemed as if it were continued outside as an external cyst (Y). This cyst had apparently pushed the operculum off, carrying it away on its head. The connection between the external and internal cysts could not be distinguished easily, but observations on subsequent outgrowths proved that it probably existed.

The fungus outside the ovum consisted of eight hyphal strands, which radiated from the external cyst. On these strands eleven sporangia were developed. Seven of the hyphae bore a single sporangium each, one bore two sporangia connected by an isthmus, while the remaining two sporangia were so close to the mother cyst that their hyphal connections were not visible. For

the sake of convenience, these sporangia have been designated A to L respectively.

Sporangium A was the first to dehisce. The sporangium measured 40μ long and 35μ wide. The dehiscence tube was 80μ in length and 5μ wide. The sporangium A was connected to sporangium B by a short isthmus 15μ long. A short, branching, mycelial filament arose from the sporangium. Sporing of A was followed in quick succession by B and C.

Sporangium B was very similar in shape to A, but in this case no indication of a nutritive mycelium could be observed. The dimensions were :—

Length of sporangium	40μ.
Width of sporangium	35μ.
Length of dehiscence tube	80μ.

Sporangium C was connected with the external cyst (Y) by a wide, strap-like, non-septate mycelium. A distinct septum marked the commencement of the sporangial swelling, another septum marked its end. Beyond the swelling the mycelium was continued as a very fine dichotomously-branched, non-septate filament. Later, a dehiscence tube was formed from the sporangium. After the discharge of the zoospores, the filamentous mycelium or rhizoids became indistinct, and eventually died away.

The dimensions were :—

Length of sporangium	37μ.
Width of sporangium	30μ.
Length of dehiscence tube	120μ.
Length of broad mycelium to cyst	100μ.

Sporangium D (Figs. 3 and 5, Pl. 13) next discharged its zoospores. A strap-like mycelium connected the sporangium with the external cyst (Y). The mycelium beyond the sporangial swelling was branching and thread-like. The dehiscence tube in this instance was peculiar, giving off a short blind branch from its base. Such a dehiscence tube was noted by Butler and Buckley, but in their case the zoospores escaped by the shorter branch, while in this case the longer branch functioned. The dimensions for D were :—

Length of sporangium	30μ.
Width of sporangium	30μ.
Length of dehiscence tube	40μ.
Length of dehiscence tube branch	20μ.
Length of strap-like mycelium	110μ.
Length of fine mycelium	95μ.

The peculiar branching of the dehiscence tube noted above also occurred in E, G, and I. In these the branches were of approximately equal length, but in each case, when dehiscence took place, one branch only functioned. (See for

E, Figs. 3 and 5, Pl. 13; for G, Figs. 3 and 6, Pl. 13; and for I, Figs. 3 and 7, Pl. 13; also the Microphotograph, Fig. 25, Pl. 18.)

Sporangia F (Figs. 3 and 5), H (Figs. 3 and 6), K and L (Figs. 3 and 4, Pl. 13) never formed dehiscence tubes. In this connection it is of interest to note that Catenaria within the ovum frequently advances to the formation of cysts when growth is suddenly arrested. This phenomenon is most common in eggs overcrowded with sporangia; most of the sporangia dehisce, but a few remain intact, and may even form dehiscence tubes, though these fail to rupture. When this stage is reached failure cannot be due to lack of sustenance, since, by the time the dehiscence tubes are formed, the zoospores are practically complete. K and L were connected by an isthmus. A mycelial filament arose from L, but curved round behind the ovum, and so could not be traced very far. The dimensions of the sporangia E to L varied from $45 \times 40\mu$ to $25 \times 25\mu$.

It was hoped that the zoospores would settle down in the medium and grow, but, although they eventually came to rest and enlarged somewhat, they failed to develop. Possibly the nutritive medium was not sufficiently concentrated, or had been entirely exhausted by the parent growth. Subsequent experiments proved that the former explanation was correct.

Second Outgrowth of Catenaria.

The second outgrowth of the Catenaria occurred while a vacation course was being conducted at the Crawford Hayes Marine Station attached to this department, and consequently it escaped notice until it had died, the medium drying up through lack of attention. The medium used was a mixture of Agar-agar, extract of fluke ova A, and white of egg flakes in equal parts. On this occasion two ova developed outgrowths into the medium, and both penetrated through the ovum wall, not through a detached opercular opening. In the first ovum two somewhat flask-shaped outgrowths, from which mycelial filaments were given off, had developed (see Fig. 8 B, Pl. 14). In the second ovum a large internal sporangium developed an outgrowth, which, after penetrating the ovum wall, formed an external cyst. This cyst gave off four mycelial strands, each bearing an elongated pear-shaped sporangium. These sporangia did not form dehiscence tubes (Fig. 8 A, Pl. 14).

The outgrowths from both ova suggest that they originated as dehiscence tubes. In neither case did the internal cyst develop any other dehiscence tube. The branching of dehiscence tubes has already been noted, and a further instance (Fig. 9, Pl. 14) in which a dehiscence tube produced three branches, and yet functioned, supports the suggestion that dehiscence tubes are primarily nutritive hyphae which have developed, secondarily, the function of discharging zoospores. The fact that these branched dehiscence tubes are more common in an artificial nutritive medium than when the fungus is growing endophytically offers support to this view, though an alternative explanation would be that

the branching is a new development correlated with an abnormal environment. The former suggestion seems the more probable. Indeed Petersen (1910, p. 498) has expressed the view that ". . . even the most primitive forms of the Chytridineae betray their relationship with the more highly organised Phycomycetes by the formation of channels for the zoospores, *a sort of mycelium* . . ."

Third Outgrowth.

The third outgrowth of Catenaria occurred in a hanging drop that was set up on August 12th, 1928. The medium used was equal parts of extract of fluke ova A, egg flakes, and tap water. The egg flakes were made by mixing two parts of egg albumen with one part of extract of fluke ova A, coagulating the mixture by heat, and forcing the mass through the fine gauze in the press already described. In this case the outgrowth developed the same day, and emerged through the opercular orifice. The outgrowth formed two main hyphae, each bearing a single sporangium. Dehiscence occurred on the 15th and 18th of August respectively (see Fig. 13, Pl. 14).

The most interesting development in this instance was the peculiar shape of one of the sporangia. No explanation associated with the cultural conditions can be advanced to account for this abnormality, as the second sporangia, practically alongside it, is globose in form. Evidently cyst shape is an extremely variable factor in Catenaria. The cysts of the first outgrowth were all globose, those of the second were all pear-shaped and elongated, while in the third outgrowth we get a typically globose form side by side with a peculiar cyst, which shows some resemblance to the elongated forms of the second outgrowth.

Cyst shape is not directly influenced, therefore, by environment, but is subject to some inner, and at present unknown, control. Lack of material could not have induced the malformation in the first case, as it is a much larger cyst than its globose fellow.

As in the two former cases, zoospores did not develop on liberation into the medium.

Although the above development took place in close proximity to the egg flakes in the medium it could not be determined that the mycelium actually extended into the flakes.

Subsequent Outgrowths.

Subsequent outgrowths occurred at intervals, but showed no noteworthy developments. Attempts made to germinate zoospores were entirely unsuccessful, though various modifications of technique were adopted, until in January, 1932, fluke ova extract B was used. In the previous media outgrowths only occurred at long intervals, and the vast majority of hanging drops gave negative results. When the fluke ova were desiccated and ground dry with kieselguhr in the preparation of the extract, the crushing was much more complete and the extract obtained was more concentrated.

On January 25th, 1932, two hanging drops were set up; the medium used contained equal parts of agar, fluke ova extract B, and water.

The same afternoon the sporangium in one of the ova was seen bulging out through the opercular orifice. The following day nine ova in one hanging drop, and eleven in the other (out of a total of 20 in each) showed vigorous outgrowths of mycelia. Unfortunately, the cover glass of the preparation containing nine ova with outgrowths was accidentally broken. Seven of these ova were successfully transferred singly to new moist chambers. One died, but all the others continued to grow, and flourished even more luxuriantly than the outgrowths in the undamaged preparation. This may be due to the absence of "staling," which Brown (1923, 1925) has described so fully, or to abundance of nutritive material.

In some cases more than twenty hyphal strands grew out from a single egg. In one instance an egg which contained three internal connected sporangia, and, therefore, presumably a single thallus, developed twenty-one main hyphal outgrowths, each bearing from three to four or more globose zoosporangia. The mycelium with its zoosporangia was clearly visible to the naked eye as a cloudy white speck; the thallus in this instance contained upwards of sixty zoosporangia (see Fig. 14, Pl. 15).

Another mycelium, which resulted from the outgrowth from a single egg, is shown in the camera lucida drawing (Fig. 15, Pl. 15). In this case it could not be determined with certainty that only one thallus was involved, though this is probable. In outgrowths through the opercular aperture, in which we were able to follow the mycelial connections, we have not found any case in which more than one thallus took part. The drawing was made from one optical plane, in which 270 sporangia were counted. At least three such planes were to be distinguished in the hanging drop, so this outgrowth probably contained over seven hundred sporangia. The drawing was made after dehiscence was completed, so the finer mycelial strands or rhizoids could not be observed.

Germination of Zoospores in Artificial Medium.

In two of these six preparations the zoospores germinated, producing profuse growths of mycelia, and advanced to zoosporangial formation.

The zoospores on escaping from the zoosporangium may swim about for some time and then settle down, or may commence to germinate quite close to the parent zoosporangium. The earliest stages observed are shown in Fig. 16 A and B, Pl. 15. The fate of the cilium has not been followed, but it was not present at this stage, though it is obvious for some time after settlement.

The zoospore grows to form an ovoid mass about 6 by 4μ, and produces a short unbranched rhizoid (Fig. 16 A′, Pl. 15). In the next stages the central mass is enlarged, and the rhizoid lengthens to 30μ (Fig. 16 B′, Pl. 16). A more advanced stage is shown in Fig. 17 A, Pl. 16; the central mass is now 14 by 10μ, and there are four rhizoids, which have commenced to branch. Three hours

later (Fig. 17 B, Pl. 16) the central mass had increased slightly in volume, and the rhizoids had become a rhizo-mycelium with four main strands. Seventeen hours later (Fig. 17 C, Pl. 16) a slight increase in volume and in the complexity of the rhizo-mycelium was noticed. Seven hours later a short dehiscence tube had developed (Fig. 18, Pl. 16). This particular thallus was stunted, and consisted only of a single sporangium. More complex thalli are shown in Figs. 19–22, Pls. 16 and 17.

Dangeard (1885), in his account of the germination of the zoospores of *C. anguillulae* on Nitella and Anguillula, stresses the bipolar character of the germination. His figures (here reproduced in our Fig. 23 C, D, E, F) show one or more hyphal threads emerging from opposite poles. He states that this character sharply distinguishes the germination of the zoospores of Catenaria from that of its near allies, Chytridium, Rhizidium, &c., in which each germinating zoospore gives rise to a hyphal filament on one side only. Karling figures the germination of the zoospores of *Diplophlyctis intestina* (Karling, 1930) of *Entophlyctis sp.* (Karling, 1931A), and of *Cladochytrium replicatum* (Karling, 1931 B). Sparrow, F. K. (1931), figures the germinating zoospores of *Nowakowskiella obscura*, n.sp. In all these cases the germination appears to be unipolar. The zoospores of Catenaria in artificial medium may show unipolar, bipolar, or multipolar germination. They appear, as far as our observations go, to be for a short period unipolar and subsequently bi- or multipolar. In contradistinction to the other genera, there are usually several axes of growth diverging from the spore, which may develop into sporangiferous filaments.

It is suggested that this multipolar germination in artificial media is a further indication of the inherent potentiality of Catenaria to develop an elaborate thallus, which it fails to display when growing parasitically.

In Fig. 21, Pl. 17, it will be observed that a sporangium has formed from a swollen hypha which has not become markedly differentiated. It measured 80μ, and the width was 7μ. This particular sporangium is strongly reminiscent of Dangeard's genus *Mitochytrium ramosum* (Dangeard, 1911), which occurs in the cells of *Docidium Ehrenbergii*, a Desmid. Dangeard's genus, however, was non-septate and more highly developed. The growth of mycelia from the germinating zoospores became matted, and extended over a considerable proportion of the hanging drop. In the tangled mass it was not possible to determine the exact number of zoosporangia formed by the thallus arising from a single germinating zoospore, but seven connected zoosporangia were counted before the overgrowth became too dense for observation.

Those zoospores which escaped into the medium after it had been colonised were less luxuriant in their growth. They settled down, became somewhat swollen, and developed a fine branching mycelium, principally from one side.

The Development of the Mycelium in Culture.

The development of the nutritive portion of the thallus of *C. anguillulae*, when growing as a parasite, is scanty, consisting of comparatively few fine

branching rhizoids. Dangeard (1885, 1886) refers to the ease with which these rhizoids may be overlooked, when the flask-shaped sporangium may be mistaken for an Olpidium.

E. J. Butler (1928) also refers to this difficulty. His description of the thallus is as follows:—

> "The thallus consists of a main axis, swollen irregularly in places and with a tendency to branch, from which arise a number of very thin walled rhizoids, given off mainly from the swollen places, but also from the unswollen parts. These rhizoids are either simple . . . or more or less branched. As in all the material examined, their fine ends could not be made out; as far as they could be traced through the egg contents they are always double contoured, and if they possess terminal branched filaments, as indicated by Dangeard, these must be exceedingly tenuous. The swollen parts figured are doubtless the places which will subsequently develop into sporangia."

When the fungus is growing in artificial media the nutritive portion of the thallus shows a remarkable development. The outgrowth into the medium consists of a number of main hyphal strands varying in thickness from 2 to 7μ, except near their termination, where they taper to fine threads.

As previously stated, over 20 such hyphae have been counted arising from a single thallus (see Fig. 14, Pl. 15). These hyphae radiate into the medium, producing lateral branches of a much finer character, the rhizoids. The latter are sharply differentiated from the main hyphae, not only by narrower diameter, but also by their abundant dichotomous branching. The main hyphae are comparatively straight, and rarely, except near the base, bifurcate into strands of equal diameter. Sorokin (1876) depicts in his Figure 24, and describes the union of three sporangia in a triangle. Dangeard denies the occurrence of such an anastomosis, and our observations confirm Dangeard's view, though sometimes such an anastomosis is simulated by the intercrossing at different levels of sporangiferous filaments.

The main hyphae appear to be at first nutritive. The growing point tapers to a very fine rhizoid-like termination, but as growth proceeds the diameter of the hypha becomes uniform. Successive stages of the development of one of these hyphae are shown in Figs. 10, 11, and 12, Pl. 14. During these phases no septa are visible. Septa appear when sporangial formation takes place (Fig. 13, Pl. 14). This may occur in two ways. The main hypha may swell to form spherical, ovoid, or irregular sporangia, between which the unthickened hypha remains as isthmuses. Alternatively, the hypha, without any marked swelling, may become segmented, alternate segments functioning as sporangia and isthmuses.

In the quotation from Butler given above, the reference to the terminal branched filaments observed by Dangeard relates to an observation made by

him when Catenaria was grown on *Nitella tenuissima*. Although he has seen branched filaments extending outside the bodies of infested Anguillulae he was unable to trace their connection with the internal sporangiferous filaments, but strongly suspected the presence of such a connection. Owing to the transparency of the medium, we have no difficulty in confirming the presence of these branched terminations. As suggested by Butler, the terminations are "exceedingly tenuous."

Karling (1930, 1931), who discusses very fully the morphology of the rhizoids in the Chytridiales, considers that the rhizoidal or mycelial system of the Cladochytriaceae is sufficiently different from that of the Rhizidiaceae to warrant the use of a special term, "the rhizo-mycelium," confining the term "rhizoid" to the Rhizidiaceae. He considers that essential differences exist between the rhizoids of the Chytrids and the mycelia of the higher fungi, and that the rhizo-mycelium of the Cladochytriaceae is a transition type between rhizoids and mycelia. The great development of the thallus of *C. anguillulae* in artificial media gives support to this view; and we submit that it approximates even more closely to the true mycelia of higher fungi than to the rhizoids of the Rhizidiaceae. In his paper of 1930, Karling states that: "Whether or not the rhizoids that are found in the Rhizidiaceae, Cladochytriaceae, and Hypochytriaceae are to be regarded as mycelia is obviously open to question. There are certain structural characteristics of rhizoids, as they have been figured, which in a general way, it seems to me, differentiate them from fungus mycelia. Almost at once rhizoids tend to decrease in diameter and run out to a fine point. The center of gravity of the growth process, so to speak, remains in the spore or in some immediate outgrowths of it, like the sporangium or apophysis."

In our cultures the main hyphae maintained their diameter for a considerable distance, though towards the termination they may taper (see Fig. 14, Pl. 15). The fact that *C. anguillulae* is capable of producing in artificial culture a more extensive mycelium tends to support the view advanced by de Bary, Sachs, and Petersen (1910)—that the Chytridiales have been derived from the higher Phycomycetes by the degenerative influence of parasitism. On the other hand, Atkinson (1909), who follows Dangeard (1901) in tracing the origin of Chytrids from unicellular organisms, such as the zoosporic Monadineae, considers that the low development of mycelium in this group is not associated with the parasitic habit. According to this view, it would appear that the poor development of the mycelium in the Chytrids is a primitive character, and that in the course of evolution the more extensive mycelium of the higher fungi developed. This view appears to be supported in substance, more or less, by most modern mycologists, though in detail they may differ. Recent writers who support this hypothesis include F. Cavers (1915), F. T. Brooks (1923), Gwynne-Vaughan and Barnes (1927), Ivimey Cook (1928), Gwynne-Vaughan (1929), Fitzpatrick (1930), and Ramsbottom (1931).

Atkinson supports his contention, by reference to the extensive rhizoidal system that is present in certain algae which have developed a parasitic habit.

He states: "It is quite clear that this extensive rhizoidal system is developed in response to a new nutritive demand. There is no evidence of degeneration through parasitism."

Since it is evident that Catenaria possesses the potentiality of producing an extensive nutritive thallus in artificial media, the question arises: Why is the potentiality not expressed when the fungus is living as a parasite? The most reasonable explanation appears to be that some factors associated with parasitism suppress the full manifestation of this potentiality. Two factors suggest themselves: in the first place, the richer and more concentrated nutritive substratum of the host may enable the necessary supply of organic material to be obtained with a less extensive thallus; or, secondly, the reaction of the living tissue of the host may exercise an inhibiting influence on mycelial development.

Turbinate Organs.

Karling (1931 B) discusses the turbinate organs of the Cladochytriaceae. Although originally regarded by Schroeter as gametes, they are now looked upon as more or less modified portions of the thallus, because they may occur in various positions apart from the resting spores. Turbinate organs are spindle-shaped enlargements of the thallus, and though usually consisting of two cells, may contain 1–6 cells. The structures have been known under various names—"Sammenzellen" (Büsgen, 1884), "Companion cell" (E. J. Butler, (1907), "cellule adjacent" (M. Cornu, 1872). The organs occur in their highest development in Urophlyctis (Jones and Drechsler, 1920, and A. W. Bartlett, 1926), and also occur in Physoderma, Cladochytrium, and Nowakowskiella. Their occurrence on Amoebochytrium is doubtful (Karling, 1931 B). Karling discusses the possibility of the occurrence of these structures on Catenaria, and states that Dangeard has figured enlargements of the thallus, particularly near the region of entry. We have failed to observe any indication of a structure resembling a turbinate organ in our cultures, either on the host or in artificial media.

Resting Spores.

Buckley and Clapham describe the occurrence of resting spores of *C. anguillulae* in cultures on *Dibothriocephalus latus* eggs. They possess a double wall, the inner one usually being considerably thicker than the outer one. An egg containing only resting spores was isolated, washed in boiled water, and added to some fresh eggs of *Fasciola hepatica*, which had been boiled to exclude any possibility of an outside infection. These eggs became infected, and both zoosporangia and resting spores were found interconnected by mycelium. These observers were working with a different strain of Catenaria (obtained from London tap-water) from that used in this laboratory.

During the past four years we had not observed the occurrence of resting spores until January, 1932, and then only in two cases. This difference of

behaviour in the two strains may perhaps be due to differences in laboratory conditions, or to the transference to a new host in the case of Buckley and Clapham's experiments.

Curtis (1921) induced the formation of resting zoosporangia in *Synchytrium endobioticum* by subjecting infected host potatoes to drought. Buckley and Clapham (1929), in one of their experiments on *C. anguillulae*, found that a sudden rise of the temperature of incubation may be followed by the formation of a high percentage of resting sporangia. In the two cases in which resting spores developed in our strain, they were within heavily infected eggs, and it was not possible, owing to opacity of the egg contents and the complexity of the thallus, to determine with certainty whether they were connected by mycelium with the zoosporangia which were also present in the same eggs (Fig. 24, Pl. 17).

J. T. Barrett (1912), who studied the development of the sexually produced resting spores in three species of Olpidiopsis, found that external conditions play a great part in the formation of these structures. By retarding the vegetative activity of the fungus in various ways he stimulated the production of resting spores. The methods employed included:

1. The use of old cultures to which new substrata were added without changing the supply of water.
2. The use of aphids as a substratum. These furnish a very limited supply of nourishment for the host fungus, and in turn for its Chytrid parasite.
3. The presence of a certain degree of Bacterial and Protozoan contamination.

Barrett's results tend to support the view that the free production of resting spores in *Dibothriocephalus latus* eggs is due to the restriction of the food supply of the thallus; these eggs being considerably smaller than those of the liver fluke.

Systematic Position.

Karling (1931), commenting on Buckley and Clapham's results, draws attention to the fact that the method of formation of the resting spores of *C. anguillulae* is not very similar to that of the other species of the Cladochytriaceae. He refers to the similarity in the position of the resting spore, relative to the remainder of the thallus, to that of many members of the Ancylistales, and indicates that, were it not for the doubtful evidence of primitive sexuality in some species, *C. anguillulae* might well, on this basis, be placed in this group. He also suggests that, until the process of oospore or resting spore formation is better known, the relation of *C. anguillulae* to this group of fungi must remain doubtful. E. J. Butler (1928) also indicates the necessity for further

study of this phase before finally allocating Catenaria to its permanent systematic position. Unfortunately, up to the present the rarity of resting spore formation has not enabled us to make any observations which throw any further light on the problem. In this connection it is well to record that we have not observed any definite indication of diplanetism in the zoospores. Zoospores are said to exhibit diplanetism when they show two periods of activity, separated by a period of rest. In the previous paper from this department (Butler and Buckley, 1927) it was noted that the zoospores sometimes emerge very rapidly, and may be held together in a ball of mucus, but this quickly breaks up, and the zoospores swim away. Atkinson (1909 (b)) traces diplanetism in a descending series from its highest expression in Saprolegnia and Leptolegnia to a reduced but distinct manifestation in *Rhizidiomyces apophysatus* (Zopf). In the latter case just prior to the formation of the zoospores an exit tube is formed through which protoplasm passes, not in a continuous stream, but in separated portions, which represent the zoospores. The protoplasm collects into a rounded vesicle at the end of the tube, and here the final differentiation of the zoospores takes place. Zopf (1884), quoted by Atkinson, says that this plant furnishes an interesting suggestion as to the hitherto unknown relationship between the Rhizidiaceae, on the one hand, and Ancylistales and Pythiaceae, on the other. In Catenaria the zoospores are definitely differentiated prior to dehiscence, but this method of outpouring and balling together may possibly represent a still further reduced case of diplanetism.

Dangeard (1885) also describes two methods by which the zoospores may be discharged in Catenaria. In one they escape singly, being deformed by their passage through the narrow dehiscence tube. In the second method several escape together surrounded by mucus, and form a ball at the mouth of the dehiscence tube. From this ball they are liberated after two seconds. His figures illustrating both methods are reproduced in our figures (23 A and B, Pl. 17). It will be seen that in the first type of dehiscence the flagellum is shown already differentiated within the sporangium. In his text he states: "... very often it is possible to see them fully formed in the interior of the sporangium." Evidently Dangeard implies that at other times the flagellum is not fully formed in the sporangium, or at least is not visible. The point is emphasised because, in his figure illustrating the second method of dehiscence, no flagella are depicted, either within the sporangium or in the mucus ball outside, though they are drawn in the zoospores that escaped two seconds later. While we have not observed any case in which the zoospores had not formed their flagella when leaving the sporangia, Dangeard's figures would perhaps give some support to the view, that in this second method of dehiscence an extremely reduced condition of diplanetism is indicated.

It is also well to record that we have not so far observed any indication of sexuality in the behaviour of the zoospores. Whether any sexual process is involved in the formation of the resting sporangium cannot be at present stated. Buckley and Clapham's Figure 12 shows a resting spore, and an empty

sporangium connected by an isthmus, but, as the sporangium has an open dehiscence tube, it is to be presumed that its contents escaped to the exterior as zoospores, and that no fusion of its contents with that of the resting spore occurred.

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Summary.

The cultivation on artificial media of *Catenaria anguillulae*, a Chytridiacean parasite on the ova of *Fasciola hepatica*, is recorded.

Successful cultures were obtained in various media containing aqueous extract of fluke ova with or without agar, and, or, egg albumen.

In culture the development of mycelium was much more extensive than when the fungus was growing as a parasite. In one instance a single thallus produced over 20 hyphal strands, each bearing, on an average, 3 to 4 sporangia, a total of over 60 sporangia being counted. On another occasion it was estimated that over 700 sporangia were present in a single outgrowth, possibly involving only one thallus. The more extensive development of the mycelium in artificial media, in the opinion of the writers, offers some support to the view that *Catenaria anguillulae* is a form reduced through the influence of parasitism.

The zoospores germinated on an artificial medium, and, in turn, produced a thallus, zoosporangia, and zoospores.

The variability of sporangial shape in culture is noted, and the morphology of the rhizoids in *Catenaria anguillulae* is discussed. The question of the occurrence of Turbinate organs and diplanetism in the species is debated, and some observations on resting spores recorded.

Description of Drawings.

The magnification is indicated in each drawing by a scale 100μ long showing 50 and 10μ subdivisions.

Fig.

1 (Pl. 13). Dehiscence of sporangium within egg of *Fasciola hepatica*, and capture of zoospores by *Actinophrys* sp.

2 (Text fig. on page 307). Press for making egg albumen shreds.

A = Boxwood piston.
B = Back flanges, bolted together.
C = Fine copper gauze.

3 (Pl. 13). First outgrowth of Catenaria, showing internal cyst X, external cyst Y, and external sporangia A–L.

4 (Pl. 13). Details of growth in neighbourhood of opercular aperture, showing detached operculum O, internal cyst X, external cyst Y, and external sporangia A, B, C, K, and L.

5 (Pl. 13). Details of external sporangia D, E, and F.

6 (Pl. 13). Details of external sporangia G and H.

7 (Pl. 13). Details of external sporangium I.

8A (Pl. 14). Second outgrowth of Catenaria, second ovum.

8B (Pl. 14). Second outgrowth of Catenaria, first ovum.

9 (Pl. 14). Dehiscence tube, with three branches.

10 & 11 (Pl. 14). Successive early stages in development of third outgrowth.

12 (Pl. 14). Shows early stage in differentiation of sporangia of third outgrowth. Finer details of mycelia are not shown.

13 (Pl. 14). Shows later stage in the differentiation of sporangia of third outgrowth. One globose and one strap-shaped sporangium have formed.

14 (Pl. 15). Outgrowth from single thallus showing 21 hyphal strands.

15 (Pl. 15). Outgrowth of mycelium from an infected ovum. Drawing made in one optical plane. Over 270 zoosporangia are figured. This drawing was made after dehiscence had occurred, and the finer rhizoids could not be seen. In some sporangia the dehiscence tubes are not visible, as they are not in the optical plane of the drawing.

Germination of Zoospores in Artificial Medium.

16 (Pl. 15). A and B, earliest stage observed of germination of zoospores. C, bipolar germination of zoospore. A′, B′, C′, later stages of A, B, C. A′ and B′ show unipolar, C′ bipolar germination.

17 (Pl. 16). A, B, C, successive stages in the multipolar germination of a zoospore.

18 (Pl. 16). Later stage of the same zoospore, which has now formed a sporangium and dehiscence tube.

19 (Pl. 16). A and B, multipolar germination of zoospore. In B the formation of a dehiscence tube has commenced.

20 (Pl. 16). The same thallus after dehiscence. The tube has curved out of the optical plane, and consequently its open end is not figured.

21 (Pl. 17). A more elaborate thallus formed from a germinating zoospore showing three sporangiferous filaments.

22 (Pl. 17). Other examples of germinating zoospores.

23 (Pl. 17). Redrawn from Dangeard (1885), Figures 5, 6, 7, and 9. *Catenaria anguillulae* growing parasitically on *Nitella tenuissima*.

A. Two sporangia, one showing dehiscence of fully formed zoospores occurring singly. At the right, a zoospore has burst through a septum, and is entering the isthmus cell. The zoospores within the sporangia are figured as having flagella.

B. Second method of dehiscence, zoospores escaping in a mass, surrounded by mucus. It is to be noted that the zoospores, both in the sporangium and in the ball of mucus, are not depicted as having flagella. The zoospores which escaped two seconds later are shown in C, and those figured in D have become attached, but are still active.

E and F show bipolar germination, early stage; F drawn on second day after dehiscence.

24 (Pl. 17). Three resting spores together with a zoosporangium in a *Fasciola hepatica* egg.

25 (Pl. 18). First outgrowth of Catenaria showing external sporangia A–L.

26 (Pl. 18). First outgrowth of Catenaria showing external sporangia B, D, E, F, and G.

27 (Pl. 18). Details of external sporangium I.

28 (Pl. 18). Details of external sporangia D, G, and H.

LITERATURE CITED.

ASHWORTH, H. J., 1922. — On *Rhinosporidium seeberi* (Wernick, 1903), with special reference to its sporulation and affinities. Trans. Royal Soc., Edin., **53**, pp. 301–342, Pls. I–V. 1922.

ATKINSON, G. F., 1909.—(*a*) Some fungus parasites of algae. Bot. Gaz., **48**, pp. 321–338, figs. 1–8. 1909.

ATKINSON, G. F., 1909.—(*b*) Some problems in the evolution of the Lower Fungi. Ann. Mycol., **7**, pp. 441–472, figs. 1–20. 1909.

BARLOW, C. H., July, 1925.—The Life Cycle of the Human Intestinal Fluke, *Fasciolopsis Buski* (Lankester). Amer. Journ. Hyg., Monographic series, No. 4, pp. 1–98, Pls. I–X, figs. 1–12. 1925.

BARRETT, J. T., 1912.—Development and sexuality of some species of *Olpidiopsis* (Cornu) Fischer. Ann. Bot., **26**, pp. 209–238, Pls. XXIII–XXVI. 1912.

BARTLETT, A. W., 1926.—On a new species of *Urophlyctis* producing galls on *Lotus corniculatus* (Linn.). Trans. Brit. Mycol. Soc., **11**, pp. 266–281, Pls. XI–XIV. 1926.

BRAUN, A., 1856.—Ueber einige neue Arten der Gattung *Chytridium* und die damit verwandte Gattung *Rhizidium*. Monatsberichte der Konigl. Preuss. Akad. d. Wissensch. zu Berlin, pp. 587–592. 1856.

BROOKS, F. T., 1923.—Some present day aspects of Mycology. Trans. Brit. Myc. Society, **9**, pp. 14–32. 1923.

BROWN, W., 1923.—Experiments in the growth of Fungi in culture media. Ann. Bot., **37**, pp. 105–129, figs. 1–7. 1923.

BROWN, W., 1925.—Studies in the genus *Fusarium*. II. An Analysis of the Factors which determine the growth-forms of certain strains. Ann Bot., **39**, pp. 373–408. 1925.

BUCKLEY, J. J. C., and CLAPHAM, P. A., 1929.—The Invasion of Helminth Eggs by Chytridiacean Fungi. Journ. of Helminth, **7**, pp. 1–14, figs. 1–21, Pl. I. 1929.

BÜSGEN, M., 1884.—Beitrag zur Kenntnis der Cladochytrien. Cohn Beitr. z. Biol. d. Pfl., **4**, Heft. 3, pp. 269–282, Pl. XV. 1884

BUTLER, E. J., 1907.—An account of the genus *Pythium* and some Chytridiaceae. Mem. Dept. Agr., India, Bot. Ser., **1** (5), pp. 1–158, Pls. I–X. 1907.

BUTLER, E. J., 1928.—Morphology of the Chytridiacean Fungus, *Catenaria anguillulae*, in Liver Fluke eggs. Ann. Bot., **42**, No. 168, pp. 813–821, figs. 1–19. (October, 1928.)

BUTLER, J. BAYLEY, and BUCKLEY, J. J. C., 1927.—*Catenaria anguillulae* as a parasite of the ova of *Fasciola hepatica*. Sci. Proc. R. Dublin Soc. (N.S.), **18**, pp. 497–512, Pls. XXIII–XXVI. 1927.

CAVERS, F., 1915.—The Inter-Relationships of the Protista and primitive Fungi. New Phyt.; **14**, pp. 94–104, 164–168, 223–227, 275–280, 302–304, figs. 1–6. 1915.

CLINTON, G. P., 1902.—*Cladochytrium Alismatis*. Bot. Gaz., **33**, pp. 49–61, Pls. II–IV. 1902.

CONSTANTINEANU, J. C., 1901.—Contributions à la flore mycologique de la Roumanie. Rev. Génér. de Bot., **13**, pp. 368–389, figs. 76–84. 1901.

COOK, W. R. IVIMEY, 1928.—The Inter-Relationships of the Archimycetes. New Phytol., **27**, Nos. 4 and 5, pp. 1–53, Pls. I–III, text figs. 1–111. 1928.

CORNU, M., 1872.—Monographie des Saprolégniées. Ann. des Sci. Natur. Bot., sér. 5, **15**, pp. 1–198, Pls. I–VI. 1872.

CURTIS, K. M., 1921.—The Life History and Cytology of *Synchytrium endobioticum* (Schilb.) Perc., the cause of Wart Disease in Potato. Phil. Trans. Royal Soc.. B., **210**, pp. 409–478, Pls. XII–XVI. 1921.

DANGEARD, P. A., 1885.—Note sur le *Catenaria anguillulae*, Sorok. Chytridium zootocum, A. Br. Bull. Soc. Linn. de Normandie, 3e sér., **9**, pp. 126–135, Pl. I. 1885.

DANGEARD, P. A., 1886.—Recherches sur les Organismes Inférieurs. Ann. de Sci. Nat. Viie sér. Bot., **4**, pp. 241–341, figs. 12–16, Pls. XI–XIV. 1886.

DANGEARD, P. A., 1911.—Un nouveau genre de *Chytridiacées*. Bull. Soc. Myc. de France, **27**, pp. 200–203. 1911.

FISCHER, A., 1892.—Phycomycetes. Rabenhorsts Kryptogamenflora, **1**, IV, Abt. Kummer, Leipzig. 1892.

FITZPATRICK, H. M., 1930.—The Lower Fungi. Phycomycetes, pp. 1–331. New York, 1930.

DA FONSECA, O., 1928.—Bol. Inst. Clin. Quirurg., **4**, p. 469. 1928.

DA FONSECA, O., and DE AREA LEAO, A. E., 1928.—Suppl. Mem. Inst. Oswaldo Cruz., **4**, 124. 1928.

GWYNNE-VAUGHAN, H. C. I., 1929. — Problems of development in the Fungi. Trans. Brit. Myc. Soc., **14**, pp. 193–201. 1929.

GWYNNE-VAUGHAN, H. C. I., and BARNES, B., 1927.—The structure and development of the Fungi. Cambridge Univ. Press. 1927.

JONES, F. R., and DRECHSLER, C., 1920. — Crownwart of Alfalfa caused by *Urophlyctis alfalfae*. Journ. Agr. Res., **20**, pp. 295–324, Pls. XLVII–LVI. 1920.

KARLING, J. S., 1928.—(*a*) Studies in the Chytridiales. I. The Life-history and occurrence of *Entophlyctis heliomorpha* (Dang.) Fischer. Amer. Journ. Bot., **15**, pp. 32–42, Pl. I. 1928.

KARLING, J. S., 1928.—(*b*) Studies in the Chytridiales. II. Contribution to the Life-history and occurrence of *Diplophlyctis intestina* (Schenk) Schroeter, in cells of American Characeae. Amer. Journ. Bot., **15**, pp. 204–214, Pl. XIV, figs. 1–2. 1928.

KARLING, J. S., 1930. — Studies in the Chytridiales. IV. A further study of *Diplophlyctis intestina* (Schenk) Schroeter. Amer. Journ. Bot., **17**, pp. 770–778, Pls. XLVI–XLVIX, 2 text figs.

KARLING, J. S., 1931.—(*a*) Studies in the Chytridiales. V. A further study of species of the genus *Entophlyctis*. Amer. Journ. of Bot., **18**, pp. 443–463, figs. 1–54, Pls. XXXV–XXXVIII. June, 1931.

KARLING, J. S., 1931.—(*b*) Studies in the Chytridiales. VI. The occurrence and life history of a new species of *Cladochytrium* in cells of *Eriocaulon septangulare*. Amer. Journ. Bot., **18**, pp. 526–557, Pls. XLII–XLIV. July, 1931.

MACNEAL, W. S., and TAYLOR, R. M., 1914. — Journ. Med. Res., **30**, p. 261. 1914.

VON MINDEN, M., 1915. — Chytridiineae, Ancylistineae, Monoblepharidineae, Saprolegniineae. Kryptogamenflora der Mark Brandenburg, **5**, pp. 209–422. 1915.

PETERSEN, H. E., 1910.—An account of Danish freshwater Phycomycetes, with biological and systematical remarks. Ann. Mycol., **8**, pp. 494–560, figs. 1–27. 1910.

RAMSBOTTOM, J., 1931.—(*a*) Aquatic Fungi. Journ. Quekett Micro. Club, **16**, pp. 151–166, figs. 1–3. 1931.

RAMSBOTTOM, J., 1931.—(*b*) Fungi Pathogenic to Man. A system of Bacteriology in relation to Medicine. **8**, pp. 11–68. 1931.

SCHRÖTER, J., 1897. — Chytridiineae. Engler and Prantl, Die Natürlichen Pflanzenfamilien, **1**, 1, pp. 64–87. Leipzig, 1897.

SERBINOV, J. L., 1907.—Beiträge zur Kenntnis der Phycomyceten. Organisation und Entwichelungsgeschichte einiger Chytridineen Pilze (Chytridineae, Schröter) Scripta Botanica Horti. Univ. Imper. Petro., Fasc. **24**, pp. 84–93, Pl. III, figs. 1–15. 1907. (In Russian, with a German diagnosis on p. 162.)

SEURAT, L. G., 1920.—Histoire Naturelle des Nématodes de la Bérbérie. Trans. Lab. Zool. Gén. Univ. Alger., p. 189. 1920.

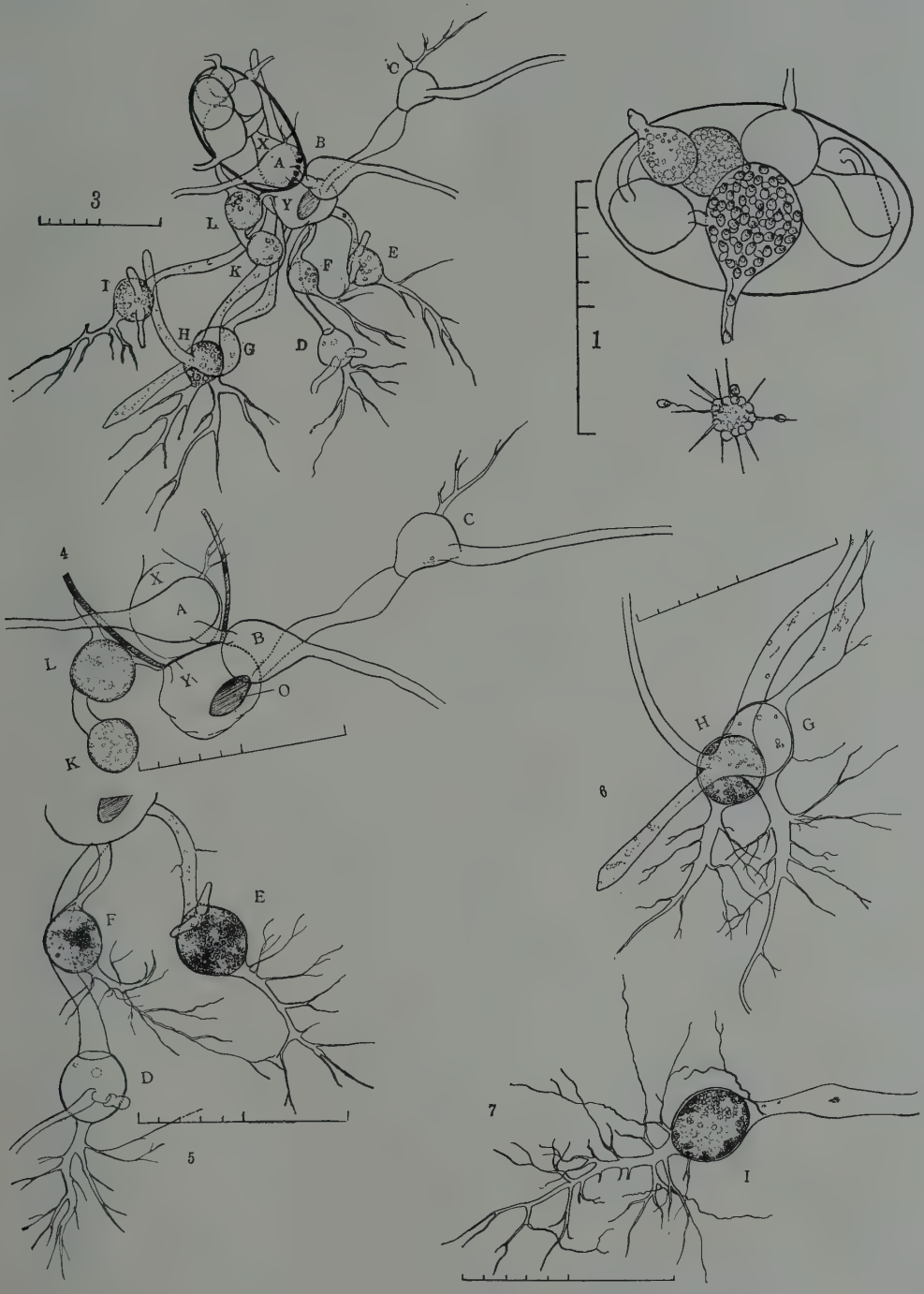
SOROKIN, N., 1876.—Note sur les Végétaux Parasites des *Anguillulae*. Ann. d. Sci. Nat., VI^{e} Sér. Bot., **4**, Pl. III, figs. 6–28, pp. 62–71. 1876.

SPARROW, F. K. (jr.), 1931.—Two new Chytridiaceous Fungi from Cold Spring Harbor. Amer. Journ. Bot., **18**, pp. 615–623, Pl. XLV. 1931.

TIRUMURTI, T. S., 1914.—*Rhinosporidium Kinealii*. Practitioner, **93**, pp. 704–719, Pls. XIV–XVI. 1914.

VILLOT, A., 1874.—Monographie des Dragonneaux. Archives de Zoologie expérimentale et générale, **3**, pp. 184–185, Pl. VI, figs. 13–18. 1874.

ZOPF, W., 1884.—Zur Kenntnis der Phycomyceten. 1. Zur Morphologie und Biologie der Ancylisteen und Chydridiaceen. Nova Acta Leop., Carol **47**, pp. 141–236, Pls. XII–XXI. 1884.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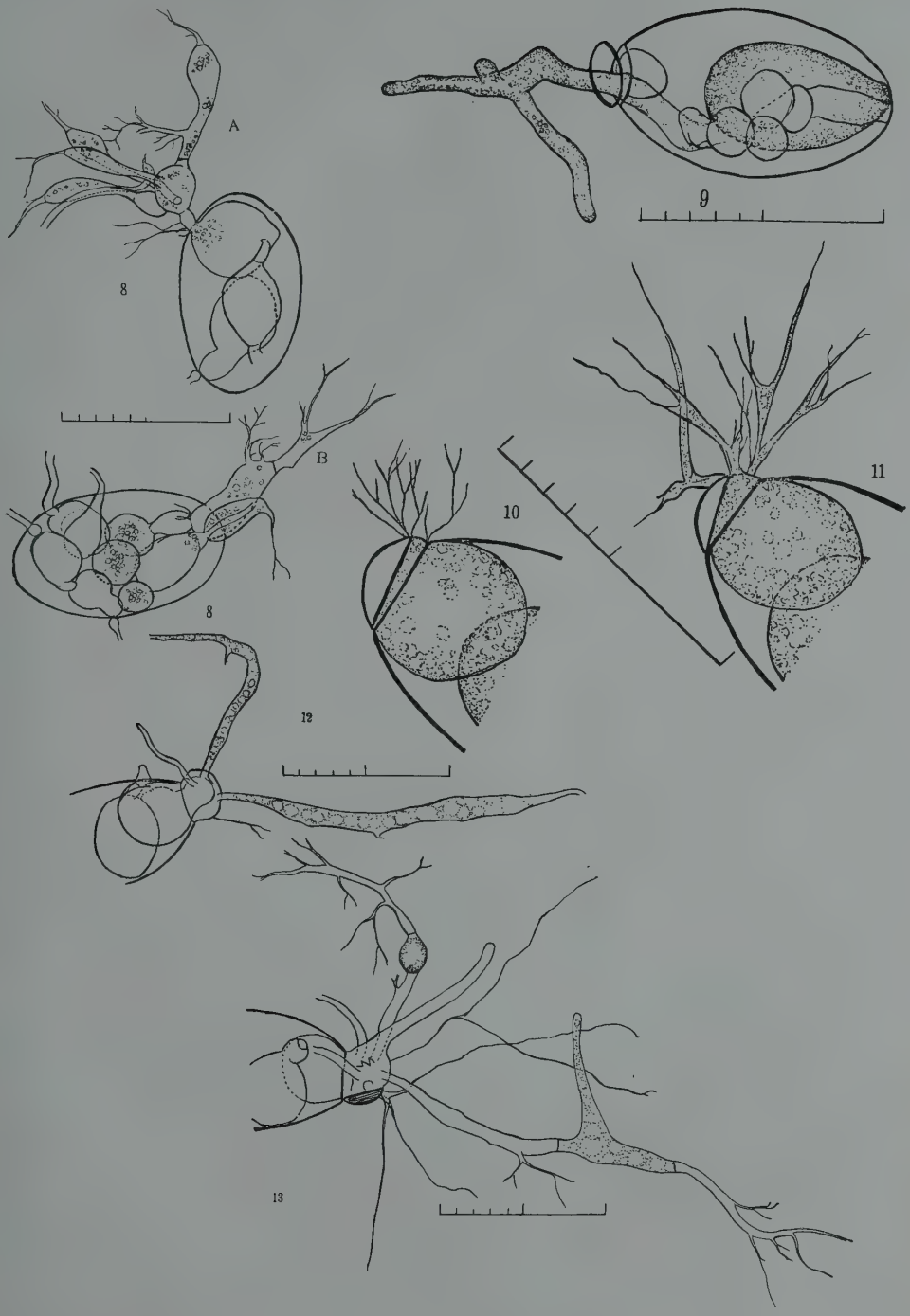
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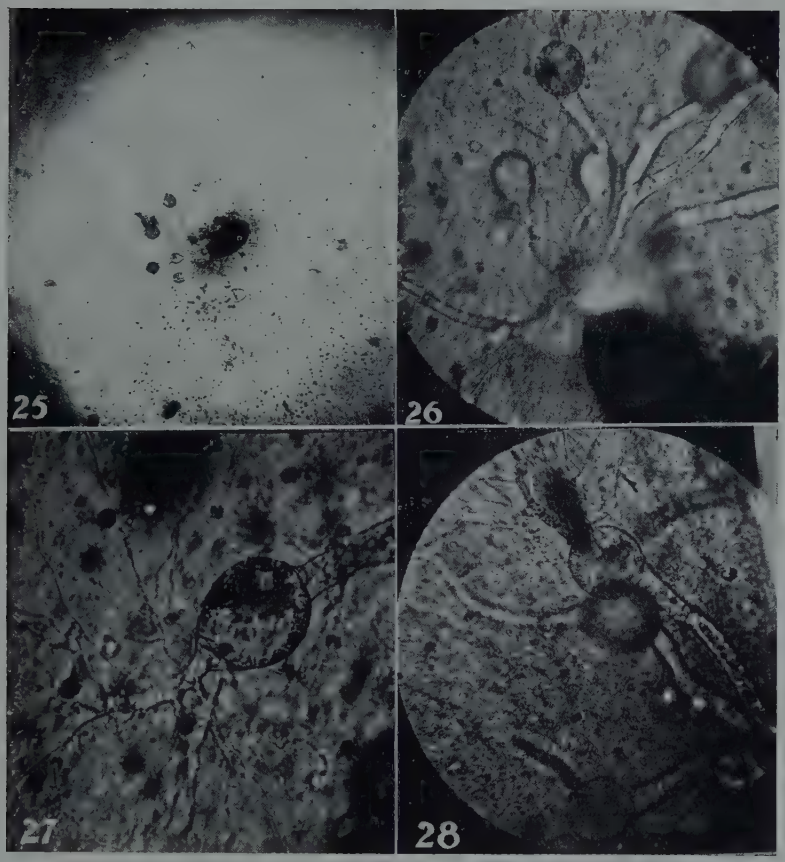
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No. 26.

FACTORS WHICH DETERMINE THE NUTRITIVE VALUE (STOCK-CARRYING AND FATTENING CAPACITY) OF UNTREATED NATURAL PASTURES.

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Pastures differ widely in their capacity to feed stock. One particular field may carry more stock per acre than another, the animals in both cases making similar progress. In that case there is said to be a difference in the stock-carrying capacity. A third pasture may carry the same number of animals as either of the foregoing, but the cattle or sheep, as the case may be, may fatten quicker, that is to say, produce a greater daily increase in weight. Thus the stock owner speaks of fattening and non-fattening pastures. The factors which make for these considerable differences in nutritive value have been the subject of much investigation, but no definite correlation has been established. It is known that such influences as soil and climate explain the comparative poverty of poor hillside and boggy pastures, and that a herbage restricted in quantity and of characteristic botanical composition is associated with such conditions. In some cases the soil is waterlogged, in others there is an excess of organic matter present, and in others still there is either an excess or a deficiency of one or more inorganic elements in the soil. In many instances, indeed, the poverty of pastures is explainable by the paucity of the herbage, due not to any single circumstance but rather to an abnormally poor soil and to adverse climatic conditions. A deficiency of some particular ingredient in the soil may be reflected in the occurrence of a similar deficiency in the herbage, thus rendering it incomplete for the support of normal development in stock. Apart, however, from these more or less exceptional cases, the cause of the difference in the nutritive value of untreated natural pastures has remained an unsolved problem.

Historical.

In an earlier paper the writer (1) brought forward evidence to show that, as between a very rich fattening pasture and a moderately good pasture not capable, however, of fattening, the difference in nutritive value could be correlated with a difference in the dry matter content of the herbage consumed by stock. No difference in the organic constituents of the herbage was determined, but the botanical composition varied, the richer pasture being pre-

dominantly grass with some clover, while the other contained a high proportion of weeds with a considerable admixture of clovers. The difference in dry matter between the mixed herbage of the two pastures was about 25 per cent. in favour of the richer, a fact which was explained by the comparative dry matter content of the different constituents which, from a number of pastures examined, worked out as follows:—

Grasses	...	26 to 32 per cent.
Clovers	...	20 to 25 „
Weeds	...	15 to 20 „

Thus the predominantly grassy herbage has a high dry matter content, while a predominantly weedy one is low therein. The figures from which the foregoing inferences were made were obtained from samples cut at various stages of the growing period, the necessity for which precaution was shown as early as 1889 by Wilson (2). Wilson in a previous paper in 1886 (3) showed that the dry matter content of clovers is less than that of grasses, and he drew attention to the importance of using grasses which have a high proportion of dry matter. Aitken in 1894 (4) and Armstrong in 1907–8 (5) attempted to correlate the high nutritive value of rich pastures with a richness of the herbage in certain chemical constituents such as nitrogen and phosphates, but other workers failed to confirm these suggestions. Among the latter are Hall and Russell (6), who, after comparing fattening with non-fattening Romney marsh pastures, concluded that the ordinary method of chemical analysis of herbage is inadequate to detect differences in nutritive value. These workers, it must be remembered, confined their analytical determinations of the herbage to the dried material, and the percentage of water in the growing sward was neglected.

Orr and his collaborators (7, 8, 9) have done a very considerable amount of work on the correlation of the inorganic content of pastures with their nutritive value, and their extensive publications, including the results of their own analyses as well as those of many workers from various parts of the world, give a great volume of data regarding the "mineral" content of pastures. It is clear that there are pastures in many countries of the world which are deficient or lacking in certain "mineral" constituents, and which, as a result thereof, are incapable of supporting normal healthy growth in stock. Definite proof that the "mineral" deficiency is responsible for the low nutritive value of the pastures is supplied by the fact that the addition of the "mineral" in question to the diet induces growth and progress. Similar results follow the manuring of the soil with an artificial fertiliser rich in the deficient element or elements. Thus cases of calcium, phosphorus, iron, and sodium deficiency in natural pastures have been demonstrated. It is clear that, in as far as these pastures are concerned, the chemical composition is an indication of their feeding value. But when pastures which, from their effects on the grazing animal, show no obvious deficiency are compared with one another the mineral composition theory of the difference in nutritive value can scarcely be accepted.

The following figures (8), which are quoted in support of the theory, do, it is admitted, show a difference in favour of the better pasture in each case, but the degree of differentiation is not significant, especially in view of the wide variation which occurs in herbage samples cut from the same pasture at different times of the year and at different stages of maturity of the plant:—

	CaO %	P_2O_5 %	Na_2O %	K_2O %	Cl %	N %	Fibre %
Good natural pasture—all grazed.	·65	·67	·37	2·66	·64	2·50	24·5
Poor natural pasture—portion eaten.	·56	·60	·41	2·60	·60	2·54	25·2
Fattening Romney Marsh pasture—portion eaten.	·99	1·01	·22	4·36	1·39	3·66	19·96
Non-Fattening Romney Marsh pasture—portion eaten.	·90	·89	·13	3·62	1·19	3·37	20·22

Besides, it cannot be claimed that the superiority of a fattening pasture over a non-fattening one, which nevertheless can produce healthy thrifty beasts of large frame, can be due to "minerals" when it is remembered that such notoriously "mineral"-deficient foods as cereals, roots, and cakes produce rapid fattening when added to a normal maintenance ration of hay.

Much of the recent work on the nutritive value of pastures has been concerned principally with the effect of certain factors of management. A considerable volume of work has been done on the effect of manuring, seeding, and method of grazing (10). Thus it has been shown that immature pasture herbage is more nutritive per unit of dry matter than mature material. The defoliation of the herbage at short intervals, however, results in a lower seasonal production than if the intervals were more extended; and, since no very appreciable depreciation of the herbage occurs up to a period of four or five weeks of growth from the previous cutting, the recommendation is made to allow an interval of that duration to elapse between successive periods of grazing. The great economic importance of these facts has been demonstrated recently by Woodman (11), Shutt (12), and Stapleton (13), but it was pointed out in 1915 by Ellett and Carrier (14), and as early as 1881 by Joulie (15). The increase in dry matter produce per acre as the interval between the periods of defoliation widens is attributed to the increased area of surface at the disposal of the plant for photosynthesis, but it is no doubt largely due also to the increase in the percentage of dry matter in the plant as it advances in age (16).[1] The application of manures raises the yield of a pasture and extends its seasonal grazing period. By it also the mineral content of the herbage may be altered (17, 18, 19). That the botanical composition influences nutritive value is well recognised. Thus the comparative richness of clovers in protein

[1] Figures given on page 336 also go to show that the percentage of dry matter in a plant increases as the plant matures.

and lime has long been known, while recent work in Wales has shown that different strains within types as well as different types of grass vary considerably in yields of dry matter (20).

Experimental.

(1) *Composition of rich and poor pastures.*

The earlier work of the writer (1) having shown no significant difference in organic composition as between an exceptionally good and a fair pasture, it was decided to investigate the composition of other pastures of well known nutritive value which had been grazed for very many years and otherwise untreated. Samples were carefully plucked from the pasture in such a way as to simulate the grazing animal; the actual portions collected having arrived at such a stage of maturity as to represent about four weeks' growth from the date of the previous plucking by the beast. Thus an average sample of the material consumed by the animal was procured, and in order to get comparable results the pastures compared were chosen as near as possible to one another, and were sampled on the same day and at a time when the stage of growth of the herbage was similar. The results obtained are shown in Table 1.

Table I.

Pasture.	Description and Location.	Percentage composition of Dry Matter.						
		Crude protein.	Ether extract.	N-free extract.	Crude fibre.	Total ash.	Lime (CaO).	Phosphate (P_2O_5).
A.	Very good, Co. Dublin	12·12	1·36	55·10	22·01	9·42	1·19	·69
B.	Mediocre, in same vicinity ...	12·74	1·12	56·35	21·50	8·30	1·28	·44
C.	Good, Co. Dublin	14·27	0·78	53·23	23·49	8·23	1·16	·52
D.	Mediocre, in same vicinity ...	14·00	1·22	54·13	22·50	8·17	1·21	·46
E.	Very good, Bruff, Co. Limerick	14·64	1·64	53·11	20·00	10·62	1·54	·72
F.	Medium (or poor), in same vicinity	12·82	1·64	55·14	21·94	8·46	1·35	·53
G.	Very good, Kilmallock, Co. Limerick	16·69	1·06	49·89	22·00	10·36	1·51	·94
H.	Poor, Kilmallock, in same vicinity	14·2	1·16	54·30	21·45	9·09	1 52	·58

The figures for pastures A, B, C, and D represent the analysis of a composite of samples successively plucked by the writer, while those from pastures E, F, G, and H show the analysis of one sample only from each pasture, kindly collected and forwarded in sealed containers by Mr. M. Gleeson, Instructor in Agriculture, Co. Limerick.

Examination of the results shows no significant difference between the first two pairs of pastures, while the richer pasture in each of the second two pairs shows a higher percentage of protein. This difference may, however, be due to a less mature condition of the herbage in the samples plucked from pastures E and G as compared with F and H, it being well known that the percentage of nitrogen in grass decreases with advance in maturity. There is a difference in the calcium to phosphate ratio of the two types of pastures due to a lower quantity of phosphate in the medium and poor than in the good samples, and, curiously, this difference, while not great in some cases, is consistent throughout. Reference will be made to this aspect of the problem later.

The following gives a rough idea of the botanical composition of the herbage of pastures G and H:—

G.—Very Good Pasture.

Rough-stalked meadow grass (*Poa trivialis* L.), large proportion.
Yorkshire fog (*Holcus lanatus* L.).
Fiorin (*Agrostis alba* L.).
Meadow foxtail (*Alopecurus pratensis* L.).
Sweet Vernal (*Authoxanthum odoratum* L.).
Perennial rye grass (*Lolium perenne* L.).
Red clover (*Trifolium pratense* L.).
White clover (*Trifolium repens* L.).
Crowfoot (*Ranunculus repens et bulbosus* L.).
Silver weed (*Potentilla Anserina* L.)
Sorrel (*Rumex Acetosa* L.).

H.—Poor Pasture.

Yorkshire fog, large proportion.
Fiorin.
Sweet vernal.
Perennial rye grass.
Crested dogstail (*Cynosurus cristatus* L.).
Hard fescue (*Festuca duriuscula* L.).
Sedge (*Carex sp.*).
Timothy (*Phleum pratense* L.).
White clover.
Red clover.
Birdsfoot trefoil (*Lotus corniculatus* L.).
Crowfoot.

Silver weed.
Narrow and wide leaved Plaintain (*Plantago major* and *P. lanceolata* L.).
Knapweed (*Centaurea nigra* L.).
Yarrow (*Achillea Millefolium* L.).
Dandelion (*Taraxacum officinale*; Web.).
Ragweed (*Senecio Jacobaea* L.).
Selfheal (*Prunella vulgaris* L.).

	Percentage of total bulk of herbage.		
	Grass.	Clover.	Weeds.
Good pasture G.	70·5	21·0	8.5
Poor „ H.	60·0	9·5	30·5

Similar analyses reported in previous work (1) credited good pastures, as compared with poor ones, with a high grass and a low weed content.

(2) *Composition of plants of the same type grown on different soils.*

The writer's earlier paper on the nutritive value of pastures was concerned principally with two pastures of well known nutritive value—one from the best fattening land of Co. Meath, and the other from a mediocre pasture in Co. Kildare. The swards from these two districts, together with the soil and subsoil, to a depth of 3 feet, were transported to the College farm at Glasnevin. When the earlier investigations were completed both sets of plots were dug and, together with some plots of Glasnevin pasture, were resown with single species of plants. Fiorin was planted in three plots on soil from Meath, Glasnevin, and Kildare, respectively, and plaintain in three others. When the plants were well established after two years' growth they were cut at intervals of four to five weeks, and the composite samples (made of equal portions of the successive cuts) analysed. The same process was repeated in the following year, and Table 2 gives the results obtained.

The Glasnevin soil is a very heavy boulder-clay type, well suited for grass, and capable of carrying a comparatively large number of stock; the Kildare soil is of the light tillage type; while that from Meath is an extremely rich heavy loam, suited for all crops, but eminently fitted for the production of fattening pastures.

Slight differences occur in the figures for the organic composition of fiorin grown on the different soils, and the same applies to the plaintain; but closer examination shows that the differences are not significant, that is to say, the composition of fiorin or plaintain, or presumably any plant, is more or less constant when grown on these three soils, which in themselves are so very divergent in character. A soil which is deficient in one or more inorganic

TABLE 2.

Description, location, and year.	Crude protein.	Ether extract.	N-free extract.	Crude fibre.	Total ash.	Lime (CaO)	Phosphate (P_2O_5).
Fiorin (*Agrostes alba* L.) on Meath soil, 1928 ...	12·63	1·38	56·95	20·83	8·21	·79	·59
,, ,, Kildare ,, ,, ...	13·28	1·74	57·82	19·81	7·35	·71	·50
,, ,, Glasnevin ,, ,, ...	13·13	1·93	53·28	22·46	9·20	·93	·71
Fiorin on Meath soil, 1929 ...	13·26	1·44	53·28	22·46	9·20	·93	·71
,, Kildare ,, ,, ...	15·68	2·02	54·41	21·00	6·93	·78	·61
,, Glasnevin ,, ,, ...	16·00	1·86	52·84	21·06	8·25	·98	·76
Plaintain (*Plantago lanceolata* L.) on Meath soil, 1928 ...	10·68	·85	67·09	10·38	11·00	3·15	·67
,, Kildare ,, ,, ...	9·89	·90	68·44	10·12	10·65	3·02	·50
,, Glasnevin ,, ,, ...	9·27	·70	68·13	10·63	11·27	3·19	·81
Plaintain on Meath soil, 1929 ...	13·37	·80	68·01	9·44	10·38	2·85	·66
,, Kildare ,, ,, ...	10·72	·70	68·13	9·00	11·45	3·74	·37
,, Glasnevin ,, ,, ...	9·83	·75	70·81	8·76	9·85	2·78	·60

elements produces a type of herbage showing a similar deficiency (21), but there is no evidence as to a direct connection between the organic matter in a plant and the chemical composition of the soil on which it grows. Manuring and treatment may alter the composition of the organic matter in a sample of mixed herbage, but that effect is chiefly due to alteration in the botanical composition. The inorganic content of the fiorin, and also that of the plaintain grown on the different soils are more or less constant, though there is a suggestion that the phosphate content of the herbage grown on the Kildare soil is lower than that of either of the others. Reference will be made to this observation later.

(3) *Digestibility of perennial rye grass, Yorkshire fog, and narrow-leaved plaintain grown on the same soil.*

A factor which suggested itself as possibly affecting the nutritive value of a pasture was the digestibility. It appeared plausible that an apparently coarse, weedy pasture of moderate carrying capacity may conceivably be less digestible than a prime fattening type. To test the suggestion directly would involve prolonged digestion experiments on the herbage of the pastures to be compared, and the precaution would have to be taken of cutting the herbage at exactly the same stage of growth in both pastures. Any possible difference in the digestibility of two such pastures would necessarily be connected with the botanical composition because of the similarity in chemical content, and, therefore, presumably, of nutritive value of the same type of plant grown on the different soils—this presumption applies to ordinary arable soils only. It was therefore decided to investigate the digestibility of three different types of plants grown under the same conditions. The plants chosen were perennial rye grass, a grass with a good reputation for pasture purposes, Yorkshire fog, a supposedly poor pasture grass, and narrow-leaved plaintain, commonly regarded as a weed. The seeds of these plants were sown, and in the second year of growth the digestibility of each plot was determined on sheep. The digestibility test was performed in duplicate, the details of the procedure being as follows: The rye grass plot was divided into fourteen divisions, a division being cut on each of fourteen successive days. Immediately afterwards the Yorkshire fog plot and, subsequently, the plaintain plot were similarly treated. The produce of this first cutting was discarded. Thirty-five days subsequent to the cutting of the first section of the rye grass plot the experiment proper was begun, the former procedure with regard to the cutting of the plots being repeated, so that the age of each section of each of the three plots, as cut, was exactly thirty-five days. The herbage cut on this second occasion was fed to the experimental animals, the first four days being regarded as a preliminary feeding period, the remaining ten days being the digestion period proper. In that way a ten-day digestion trial was made on each of the three plants cut at the same stage of growth. The following are the figures for the analysis of the herbage, the quantity consumed, and the percentage digestibility:—

Percentage Composition of dry matter (composite samples of portions taken each day during the 10-day experimental period).

	Crude protein.	Ether extract.	N-free extract.	Crude fibre.	Total ash	Lime (CaO).	Phosphate (P_2O_5).
Perennial rye grass (*Lolium perenne* L.) ...	13·08	1·79	49·78	23·20	12·15	1·24	·88
Yorkshire fog (*Holcus lanatus* L.)	13·75	1·92	45·83	24·56	13·94	1·09	1·17
Plaintain (*Plantago lanceolata* L.)	8·65	1·15	58·70	18·19	13·31	3·31	·91

Quantitative consumption of herbage per day.

		Green material. (Grams.)	Dry matter. (grams.)	Percentage of dry matter in green material.
Rye grass	Sheep I	5,000	1,252·7	25·05
	Sheep II	5,000	1,252·7	
Yorkshire fog	Sheep I	5,000	665·4	13·3
	Sheep II	5,000	665·4	
Plaintain	Sheep I	5,000	762·0	15·25
	Sheep II	5,000	762·0	

Percentage digestibility of the dry matter consumed.

		Dry matter.	Organic matter.	Crude protein.	Ether extract.	N-free extract.	Crude fibre.
Rye grass	Sheep I	63·05	67·97	53·44	18·91	69 04	73·02
	Sheep II	63·46	68 06	54·17	18·32	69·20	72·74
Yorkshire fog	Sheep I	66·86	70·30	69·18	19·63	67·36	77·29
	Sheep II	66·42	70·25	69·00	20·98	67·02	76·05
Plaintain	Sheep I	65·48	69·16	39·62	48·06	72·66	70·94
	Sheep II	65·79	69·69	38·25	46·69	73·75	70·65

The herbage was cut each morning and strewn on a concrete floor for twenty-four hours before being weighed out for feeding. It was usually damp either from dew or rain at the time of cutting, and the object of the interval between cutting and feeding was to give an opportunity for some of the surface water to disappear. The quantity of green material consumed was gauged roughly by the appetite of the sheep. The quantity of dry matter fed during the successive days of each digestibility test varied to some extent because of the daily variation in the percentage of dry matter in the green material fed, and the figure recorded is the average for the experimental period in each case. Attention is drawn to the great difference in the actual quantity of dry matter ingested from the same weight of green herbage in the case of the three plants fed. This is obviously due to the considerable difference in the dry matter of the fresh herbage, a point to which reference will be made later.

The composition of the dry matter shows a remarkable similarity between perennial rye grass and Yorkshire fog; plaintain is much lower in protein, fat, and fibre, and proportionately higher in nitrogen-free extract than the grasses: it also shows a much higher lime content. In this respect the figures agree with the data for the comparative dry matter composition of plaintain as compared with fiorin in the previous section. It is interesting to note that the composition of the dry matter of a similar weed, namely, dandelion, as given by Shutt (22), shows a low content of fibre and a very high proportion of total ash, presumably containing much lime.

The digestibility figures show remarkable agreement throughout. The two sheep digested the herbage equally well, and the proportion of dry organic material which was digested in the case of each plant worked out at approximately 70 per cent. It is clear from these results that plants so widely different from one another as good pasture grasses, poor pasture grasses, and weeds, provided they are cut at about the same stage in maturity, do not differ from one another in digestibility by the ruminant animal.

(4) *Dry matter content of pasture herbage.*

A determination of the comparative dry matter of different pastures necessitates special conditions. The pastures must be situated in close proximity so as to ensure that they are cut under exactly similar weather conditions, that the cutting is performed to the same degree of closeness, and that they are cut at approximately the same time. The herbage to be compared must be at the same stage of maturity, and obviously there must be no surface moisture on the leaves or stems at the time of cutting. Accordingly, in the dry matter determinations, only the contiguous plots referred to in previous sections were used, and the defoliation was performed in the afternoon of a dry day succeeding a dry night. The herbage was transferred without delay into large weighed bottles, which were immediately sealed to prevent loss of moisture, and the increase in weight of the bottle gave the weight of green herbage. The material was air dried for some time, and subsequently dehydrated

in a steam oven till the weight became constant. The data on Table 3 were collected from the plots referred to in section 2, and those on Table 4 from the plots dealt with in section 3.

TABLE 3.

	Description, location, and date.	Percentage Dry Matter.
Fiorin on	Meath soil, cut 23.5.'28	30·8
,, ,,	Kildare ,, ,, ,, ...	31·4
,, ,,	Glasnevin ,, ,, ,, ...	30·3
Plaintain ,,	Meath ,, ,, ,, ...	19·0
,, ,,	Kildare ,, ,, ,, ...	18·4
,, ,,	Glasnevin ,, ,, ,, ...	22·4
Fiorin on	Meath soil, cut 6.7.'28	32·9
,, ,,	Kildare ,, ,, ,, ...	33·5
,, ,,	Glasnevin ,, ,, ,, ...	32·4
Plaintain ,,	Meath ,, ,, ,, ...	18·4
,, ,,	Kildare ,, ,, ,, ...	19·8
,, ,,	Glasnevin ,, ,, ,, ...	22·6
Fiorin on	Meath soil, cut 20.8.'28	32·8
,, ,,	Kildare ,, ,, ,, ...	33·5
,, ,,	Glasnevin ,, ,, ,, ...	34·8
Plaintain ,,	Meath ,, ,, ,, ...	20·4
,, ,,	Kildare ,, ,, ,, ...	22·5
,, ,,	Glasnevin ,, ,, ,, ...	20·4
Fiorin on	Meath soil, cut 14.6.'29	28·0
,, ,,	Kildare ,, ,, ,, ...	27·7
,, ,,	Glasnevin ,, ,, ,, ...	30·5
Plaintain ,,	Meath ,, ,, ,, ...	18·7
,, ,,	Kildare ,, ,, ,, ...	17·4
,, ,,	Glasnevin ,, ,, ,, ...	19·7
Fiorin on	Meath soil, cut 16.7.'29—4 weeks after application of nitrate of soda.	21·2
,, ,,	Kildare ,, ,, ,, ,,	21·9
,, ,,	Glasnevin ,, ,, ,, ,,	20·6
Plaintain ,,	Meath ,, ,, ,, ,,	15·3
,, ,,	Kildare ,, ,, ,, ,,	14·9
,, ,,	Glasnevin ,, ,, ,, ,,	15·1
Fiorin on	Meath soil, cut 26.8.'29—Second cutting after application of nitrate of soda.	22·0
,, ,,	Kildare ,, ,, ,, ...	21·4
,, ,,	Glasnevin ,, ,, ,, ...	21·7
Plaintain ,,	Meath ,, ,, ,, ...	16·8
,, ,,	Kildare ,, ,, ,, ...	15·4
,, ,,	Glasnevin ,, ,, ,, ...	15·9

TABLE 4.

			Percentage Dry Matter.
Rye grass	determination (1)	Cut at stage most suitable for grazing.	27·1
,, ,, ...	,, (2)		28·0
,, ,, ...	,, (3)		28·2
Yorkshire fog	determination (1)		22·3
,, ,, ...	,, (2)		21·9
,, ,, ...	,, (3)		19·4
Plaintain	determination (1)		13·4
,, ...	,, (2)		15·2
,, ...	,, (3)		14·8
Rye grass	determination (1)	Cut when the herbage approached the hay-making stage.	33·5
,, ...	,, (2)		33·3
Yorkshire fog	determination (1)		26·2
,, ,, ...	,, (2)		25·9

It is clear from the perusal of Tables 3 and 4 that the same plant growing on Meath, Kildare, and Glasnevin soils has approximately the same dry matter content, but that the various plants which constitute a pasture sward differ from one another considerably in their content of dry matter. A hundred pounds of green fiorin fresh from the pasture, and free from adhering surface moisture, contains 50 per cent. more dry matter than the same weight of plaintain. Similarly, a unit weight of perennial rye grass from pasture herbage contains about 33 per cent. more dry matter than the same weight of Yorkshire fog, and approximately 66 per cent. more dry matter than the same weight of plaintain cut under the same conditions. The tables also show that the more mature herbage is richer in dry matter than the younger material.

The effect of the application of a quick-acting nitrogenous fertiliser is interesting: coincident with an increase in the quantity of the herbage there was a decided fall in the dry matter of both the fiorin and plaintain, so that the actual total quantity of dry matter produced per acre does not rise proportionately with the increase in the weight of herbage.

DISCUSSION.

Chemical Composition.

With the exception of protein and phosphorus no outstanding difference in the chemical composition of the dry matter appears as between very good and mediocre or poor pastures. In some instances there appears to be a significant difference in the proportion of protein, but this is not apparent in all cases. The percentage of protein in plaintain and dandelion, and presumably in other weeds, is considerably lower than that of grass, and consequently a high proportion of weeds in a pasture is responsible for a low percentage of protein in the mixed herbage thereof. There is no evidence of a difference in the protein content of a particular plant collected from good and bad pastures at the same stage of growth.

The percentage of phosphates, however, brings out a difference not alone as between the mixed herbage of good and mediocre pastures, but also as between the same type of plant grown on different soils—see Tables 1 and 2. The difference in some cases is not very striking, but the consistency of its occurrence prevents its dismissal without comment. Further analysis of the grass, clover, and weed portions of rich and poor pastures from the Kilmallock area show some very interesting figures in this connection :—

	Lime (CaO).	Phosphate (P_2O_5).	Total Ash.
	%	%	%
Rich pasture—grass portion	1·01	·95	10·45
Poor ,, ,, ,, ...	1·18	·49	8·9
Rich pasture—clover portion	2·25	·86	10·82
Poor ,, ,, ,, ...	2·5	·49	10·25
Rich pasture—weed portion	2·2	1·06	11·58
Poor ,, ,, ,, ...	2·0	·50	9·52

Aitken's (4) figures suggest a similar correlation between the nutritive value of pastures and the available phosphate of the soil. While the figures presented in this paper indicate a relationship between the nutritive value of a pasture and its phosphate content, there is no evidence that the smaller proportion of phosphate in the one type of pasture, as compared with another, is responsible for its lesser nutritive value. Besides, the relation may not hold, and apparently does not hold, in the case of other comparable pastures of high and low carrying capacity, and evidence is brought forward in this paper to show that the

difference in nutritive value is explainable on other grounds. Yet the phosphate content of herbage in this connection offers a subject for further experimental work.

Digestibility.

The digestibility of herbage is obviously affected by the stage of maturity, on which depends the amount of indigestible material present. Digestibility comparisons must, therefore, be made on herbage at the same stage of maturity. When this is done it is found that such diverse types as perennial rye grass, Yorkshire fog, and plaintain are similarly digestible. The results of Woodman (23) show that creeping bent grass is no less digestible than supposedly superior grasses when cut at the immature stage of growth. Results reported in this paper show that the difference in nutritive value of pastures cannot be attributed to digestibility. One occasionally hears of an exceptionally rich fattening pasture being "too rich for young stock" as an explanation of the fact that on such pastures young cattle make less progress than aged ones. Apparently the differential rate of progress of young and advanced cattle on this type of land is merely a question of management. Such pastures grow so rapidly and are so very concentrated that, unless they are very heavily grazed, they outgrow young stock; and because of the increasing maturity of the surplus herbage the digestibility is lowered, and the cattle accordingly make less progress than under conditions of closer grazing. Older cattle are more capable of keeping the growth of herbage, under such fertile conditions, in check.

Dry Matter and Botanical Composition.

What, then, is the explanation of the very considerable difference in the nutritive value of pastures? Previous work (1) supported by data from the present investigation goes to show that, when pasture herbage is segregated into the grass, clover, and weed portions, the proportion of grass is highest in the good pastures and lowest in the poor ones, while the proportion of weeds is in the reverse order. Clovers, while present, are never in very high proportion in prime pastures, while they are frequently abundant in mediocre ones. As already mentioned, grasses, clovers, and weeds show an extraordinary difference in dry matter, grasses being highest, weeds lowest, and clovers intermediate in this respect. The connection between this fact and the nutritive value of a pasture may not be evident at first sight, but when it is realised that the animal's food tube is limited in capacity it is clear that only a certain volume or bulk of herbage can be ingested daily. It is obvious, therefore, that a type of herbage which, in a unit bulk, contains a certain quantity of dry matter is superior in nutritive value to another type which, in the same bulk of green material, contains less dry matter. Presumably a definite weight of green herbage from pastures differing in botanical composition, but cut at the

same stage of maturity, occupies the same bulk in the alimentary tube, and hence the dry matter per unit weight of herbage is indicative of the nutritive value of the pasture. This is forcibly brought out in the figures which give quantitative consumption of material by the experimental sheep, page 333. The animals, eating to repletion under the experimental conditions, consumed 5,000 grams of fresh green rye grass, Yorkshire fog, and plaintain, but the ingestion of dry matter was actually 1252·7, 665·4, and 762 grams, respectively. The relationship between the dry matter content and the nutritive value of different varieties of roots has long been recognised, and when the farmer endeavours to explain that the milk production of his cows on pasture over an exceptionally wet period is no greater than in average weather, notwithstanding a much increased growth of grass, he is correct in attributing the fact to an excessively watery condition of the herbage. For a similar reason the increased produce of green herbage after a dressing of nitrogenous manure is not capable of supporting an increase in stock numbers comparable with the increase in the produce of herbage. A considerable amount of surface water accumulates on pasture herbage as rain or dew. Hughes (24) gives a series of dry matter figures for grass which range from 23 to 31 per cent., while his figures for similar grass cut on wet days approximate to 16 per cent. While a variation in the amount of water in the plant cells does not upset the relationship between the weight of herbage and the bulk of same in the stomach, water adhering to the surface increases the weight of herbage without necessarily increasing, to any considerable extent, the space which it occupies in the food tube. This is due to the rapidity with which surplus free water can pass away from the stomach into the intestine, leaving the herbage behind it. Hence the presence of rain or dew does not in itself seriously depress the feeding value of pasture herbage, except, perhaps, in the case of sheep, which require so little water: should the rate of progress or of production slow down during a period of continued rain, the effect is attributable principally to the increase in actual water content of the plant cells rather than to surface water.

Table 3 shows that the dry matter content of a particular type of plant growing on different soils does not vary, but that the different types of plant which constitute a pasture herbage show a very considerable variation in that respect. Hence the botanical composition is indicative of the percentage of dry matter in a pasture and thereby of the nutritive value of a unit weight of herbage therefrom. Generally speaking, weeds, particularly of the broad-leaved variety, are very low, while grasses are high in dry matter. But the individual grasses vary considerably in this respect also, and a comprehensive investigation into the dry matter content of all pasture grasses should provide very useful results. Clovers are, in respect of dry matter, intermediate between grasses and weeds, being definitely inferior to grasses. Hence a pasture which is predominantly clover contains less dry matter per unit weight of herbage and per unit bulk of herbage than a predominantly grassy one. It is correspondingly less nutritive. Clover, however, serves a very special purpose in

a pasture through the agency of its nodule bacteria which synthesise from the air nitrogenous compounds that fertilise the surrounding grasses. Hence the special use of a moderate amount of clover in a pasture. Clover is also very rich in protein and in lime.

Incidentally, the results provided in this paper lead to the inference that, because the digestibility of different plants and different pastures cut at the same stage of maturity is similar, the weight of dry matter produced per unit area may be confidently used as a measure of the food production of a pasture.

Density and rate of growth.

On the presumption that the ground is all covered, the proportion of narrow- to broad-leaved plants determines the density of a pasture, which in turn determines the weight of green herbage obtainable from it per unit area. Grasses are essentially narrow-leaved, clovers and weeds are generally broad-leaved, the weeds varying very much in this respect, such varieties as daisies and broad-leaved plaintain being extremes. It is clear, therefore, that the proportion of grasses to broad-leaved plants present determines the density of the pasture. The sward of prime fattening land is constituted by a dense mat of narrow-leaved grasses, together with a moderate or small amount of clovers, the herbage being so close as to feel, when walked on, like the pile of a luxurious carpet. At the other extreme is the weedy pasture on a light soil in which the broad leaves of such plants as plaintain cover a considerable area of ground, and where the proportion of grass is low. Obviously in this case the weight of green herbage per unit area is very much less than in the other, and, of course, the percentage of dry matter in the herbage is less also. Woodman (25) lays emphasis on the importance of density and luxuriance of herbage and on the effect of botanical composition. The question arises as to why one type of soil favours herbage of a type to give rich pastures while another favours less valuable herbage. There are obviously certain factors of soil texture and composition which are responsible, an investigation into which would probably open up a field of information which may lead to very great advance in the technique of pasture treatment.

The rate of growth of the herbage determines the length of interval which must elapse between successsive periods of grazing. A soil which produces quicker growth than another favours, apart from any other circumstances, a more nutritive pasture. A notable instance of rapid growth is the case of "crag," a type of pasture which occurs on shallow soil overlying a limestone rock rich in phosphates in north Clare and Galway. The "crag" is a very palatable and a predominantly grassy pasture, which is kept closely grazed by stock, and which appears to spring up in a night like mushroom. Pastures exhibit very considerable difference in the rate of growth, and the physical, chemical, and biological factors which make for this differentiation should form the subject of a fruitful piece of investigation.

Palatability.

The ready consumption of herbage depends on its palatability, and it is notable that some of the most nutritive of pastures are very palatable. The Welsh Plant Breeding Station at Aberystwyth has shown that individual grasses differ in this respect, and it is well known that many of the miscellaneous plants met with in certain types of pastures are left uneaten even by hungry stock. There is no evidence, however, that the palatability of an individual type of plant grown on different soils varies, if such cases as paddocks near farmyards, where constant if not intentional treatment may operate, are excepted. Yet the possibility of such a variation in palatability is not definitely excluded, but, generally speaking, the botanical composition determines the palatability, and a definite knowledge of the conditions which determine the flora of a natural pasture would enable palatability problems in most cases to be solved.

Stock carrying and fattening capacity.

To the farmer the nutritive value of a pasture implies (*a*) the number of cattle or other stock which a unit area can carry over a season, and (*b*) the rate at which the beasts thrive. One pasture may carry more stock than another, but the animals in both may thrive equally well. On the other hand, two pastures carrying the same number of stock may differ in so far as the cattle on one may fatten more rapidly than on the other. The two factors operate collectively in the case of two comparable pastures, one of which supports more stock than the other, and produces, at the same time, a greater rate of increase—fattening—per individual animal. On the assumption of a plentitude of herbage in both cases the question arises as to the cause of the differentiation in rate of fattening on two pastures. A difference in water content is capable of effecting the differentiation. Supposing, for instance, that a bullock receiving a few pounds of hay is fed *ad libitum* with roots. If the roots contain 10 per cent. of dry matter a certain rate of gain is made by the animal, but if the 10 per cent. dry matter roots are replaced by another variety containing 20 per cent. dry matter, and the latter are, as before, fed *ad libitum* together with the same hay allowance as previously provided, the rate of progress and the increase in weight of the animal become much greater. Similarly, the pasture herbage which contains more dry matter than another is capable of effecting greater progress and a quicker rate of fattening. The fattening pasture must contain a high percentage of dry matter, and must, therefore, contain herbage which is predominantly grassy. Furthermore, the herbage must be close, so that the animal is not required to expend too much labour in collecting its food. The optimum condition is where the beast can fill itself to repletion with concentrated herbage in a short period, and then retire to rest and ruminate.

Stock carrying capacity is, of course, affected by the dry matter of a pasture as well as by its density, but it is also affected by the rate of growth.

A pasture may have a high stock carrying capacity and yet may not have high fattening capability. This is explainable by the fact that the herbage, though abundant, may not be sufficiently concentrated to allow the animal to ingest sufficient dry matter daily to foster rapid deposition of fat.

While the comparative fattening capability of two pastures is obtainable to a fair degree of accuracy by the use of animals of the same type and weight, the determination of the comparative stock carrying capacity is rather more complicated. When two pastures of equal areas carry different numbers of stock, presumably of similar type and weight, and when the daily increase in weight is unequal, the quantity of herbage which is responsible for maintenance and for increase in weight must be compounded. So difficult is this that some investigators prefer to use number of animal-grazing days as a criterion of the value of a pasture, but here again obvious difficulties arise. If the comparable animals on the pastures under comparison increase at a different rate, and should one pasture be grazed a little more closely than another, then the number of animal-grazing days would not exactly represent the nutritive value. The writer is of opinion that the total seasonal production of dry matter of herbage cut at intervals is a more correct criterion of the nutritive value of pastures than are figures obtained from the grazing of stock thereon. In this connection great care should be taken not to cut the herbage too bare, at any rate not closer than an animal on a wide pasture range would pluck it. Findlay (26), working with cattle, found that rotational grazing gave him poorer results than did continuous grazing, but he interpreted the result as being due, not to the rotation of cattle among the plots, but to the fact that the rotational grazed plots were cropped too tightly, more especially at the beginning of the year.

Features connected with the nutritive value of natural untreated pastures requiring further investigation.

The work reported in this paper is concerned with ordinary lowland pastures, and does not concern such extremes as bog grazing, mountainous pastures, and other more or less exceptional types. Much work has been done on mountainous pastures, and in many cases a "mineral" deficiency of the soil has been shown to operate as a limiting factor to the growth and production. In many parts of the world lowland pastures in restricted localities also suffer from "mineral" deficiency. In Ireland the breed of sheep evolved on some of the mountain pastures is much smaller than the lowland types. This is particularly noticeable in the case of the Wicklow sheep, on the Leinster Chain of Mountains, and the Kerry sheep, on the Kerry uplands. There may possibly be a partial explanation of this fact in some "mineral" deficiency of the soil and herbage. Apart from any such deficiency the sparsity of herbage would, of course, give an explanation of the low nutritive value. In fact sparsity of herbage, and consequently insufficient food intake, may result in pronounced

evidence of deficiency of one or more of the "mineral" or other ingredients of herbage, whilst a greater consumption of the same type of herbage may prevent the occurrence of any such disease condition. Thus the writer found that, when chicks, kept in the absence of direct light, ate a certain quantity of food containing a small proportion of cod liver oil, they seemed to develop more or less normally, while, when the consumption of the same mixture was reduced, the intake of cod liver oil was insufficient to prevent the development of a rickety condition.

The further problems calling for investigation concerning the nutritive value of pastures which arise out of the work reported in this paper and the discussion thereon are as follows:—

(1) The possible connection between certain factors which influence the nutritive value of pastures and the percentage of phosphate in the herbage.

(2) The comparative dry matter content of the various types of grasses and of the various types of weeds which enter into pasture complexes.

(3) The physical, chemical, and biological factors of soils which favour the production of diverse types of plant, and hence herbage of dissimilar dry matter content and density, in different pastures.

(4) The physical and chemical factors of soils which cause a differentiation in the rate of growth of the herbage of different pastures.

(5) The investigation of the carrying capacity of mountainous and boggy pastures from the points of view of chemical composition, digestibility, dry matter in conjunction with botanical composition, density, rate of growth, and palatability, and the possible operation of other factors in the case of these pastures.

ACKNOWLEDGMENT.

The writer wishes to acknowledge gratefully the assistance rendered him in the chemical analysis of the herbage examined in this investigation by Mr. B. J. Senior, M.SC., of the Animal Nutrition Department.

SUMMARY.

1. Results are reported of an investigation into (1) the chemical composition of the herbage from poor and rich pastures, (2) the composition of the same plants grown on different soils, (3) the digestibility of a good and an indifferent grass and of a weed, and (4) the dry matter composition of pasture herbage: the investigation was concerned with ordinary lowland pastures of average type.

2. There was no consistent difference in the chemical composition of the dry matter of the organic portion of the herbage of very good and mediocre or poor pastures from the same neighbourhood; in certain cases the richer

pasture was higher in protein, but this fact is explainable by the higher proportion of grass to weeds therein as compared with the poorer pasture: the protein content of grasses is higher than that of weeds.

Fiorin (*Agrostis alba* L.) grown on three types of soil, one taken from an excellent fattening pasture, a second from a less rich but yet a pasture of very high stock carrying capacity, and a third from a mediocre pasture, did not show any consistent difference in the chemical content of the dry matter: the composition of plaintain grown on these three soils was also similar.

The inorganic analysis showed no difference in the lime content of the different types of herbage compared, but there was a consistent, though not pronounced, inferiority in the phosphate content of the poorer as compared with the richer type of herbage: similarly, individual types of plant grown on the soil of a pasture of lower carrying capacity contained less phosphate than the same type of plant grown on soil from superior pastures. It cannot, however, be concluded that the disparity in phosphate content is responsible for any variation in the nutritive value of pastures.

3. The digestibility of three very different types of pasture plant—perennial rye grass, Yorkshire fog, and plaintain—was shown to be similar, and it is concluded that existing differences in the nutritive value of pastures cannot be attributed to digestibility.

4. The percentage of dry matter in a pasture herbage is indicative of the nutritive value of a unit weight thereof.

The dry matter content, and hence the nutritive value, can in this connection be correlated with the botanical composition, grasses being highest in dry matter, weeds lowest, and clovers taking an intermediate position. In this respect also, the grasses which enter into a pasture herbage differ from one another.

The disappointing results which follow the consumption of the luxurious herbage produced by nitrogenous manuring or continued heavy rains are explained by a lowering of the dry matter content of the herbage.

5. The total weight of dry matter per unit area may be confidently used as a measure of the food produced by a pasture.

6. The density of a pasture, which is indicative of the weight of green herbage per unit area, is determined by the proportion of narrow-leaved plants (grasses) to broad-leaved ones (weeds and, to a lesser extent, clovers) present. Some indefinite physical, chemical, and biological soil factors are responsible for the specificity of flora in the varying types of pasture.

7. Apart from other factors, the rate at which herbage grows determines the amount of food made available to stock, and the operation of this factor is most pronounced in the case of closely grazed pastures.

8. The fattening capacity of a pasture is determined by the dry matter content of the herbage and by its density: a very close mat of concentrated herbage enables the beast to feed to repletion with the minimum of expenditure: a pasture whose herbage is high in dry matter is concentrated in nature, and

therefore the amount of green herbage which fills the food tube of a beas contains in that case more nutriment than a similar amount of herbage having a lesser percentage of dry matter.

9. A pasture may not be of a fattening type and yet may have a high stock carrying capacity, because the herbage, while not sufficiently concentrated, may be abundant, due to a quick rate of growth. Stock carrying capacity is therefore determined by the rate of growth of the herbage as well as by its density and dry matter content.

10. As measures of the comparative nutritive value of pastures the progress made by stock, the number of animal-grazing days, and the total weight of dry matter cut at intervals are discussed.

11. A list of problems connected with the nutritive value of pastures which call for further investigation is given.

REFERENCES.

1. SHEEHY.—"The correlation of nutritive value with dry matter content of pastures." Sc. Proc. Roy. Dub. Soc., **18** (N.S.), p. 389. (1927.)
2. WILSON.—"The nutritive value and produce of some grasses and clovers." Trans. High & Agr. Soc., fifth series, **1**, p. 1. (1889.)
3. WILSON.—"The nutritive value and produce of some permanent grasses" Trans. High & Agr. Soc., fourth series, **18**, p. 148. (1886.)
4. AITKEN.—"Does chemical analysis afford a reliable indication of the feeding value of a pasture?" Trans. High & Agric. Soc., fifth series, **6**, p. 410. (1894.)
5. ARMSTRONG.—"The Botanical and Chemical composition of the herbage of grasses and meadows." Jr. Agr. Sc., **2**, p. 283. (1907–8.)
6. HALL AND RUSSELL.—"On the causes of the high nutritive value and fertility of the fattening pastures of Romney Marsh and other marshes in the S.E. of England." Jr. Agr. Sc., **4**, p. 339. (1911–12.)
7. ORR.—"The nutritive value of pastures from the point of view of the mineral content." Grassland problems, Br. Min. Agr. Misc. Pub., No. 60, p. 5. (1928.)
8. ELLIOTT, ORR, AND WOOD.—"The mineral content of pastures." Scottish Jr. Agric., **8**, No. 4. (Oct., 1925.)
9. ORR.—"The relation of the chemical composition of pasture to its feeding value." Trans. High & Agr. Soc. Scot., fifth series, **41**, p. 99. (1929.)
10. FAGAN.—"The nutritive value of pasture grass as influenced by management." Welsh Jr. Agric., **5**, p. 99. (1929.)
11. WOODMAN, NORMAN, AND FRENCH.—"Nutritive value of pasture." VII. Jr. Agric. Sc., **21**, p. 267. (1931.)
12. SHUTT.—Report of the Division of Chemistry, Dept. of Agriculture, Ottawa. Year ending 31st March. (1930.)

13. Stapleton.—"Characters which determine the economic value of grasses." Jr. Min. Agric., **34**, p. 146. (1927.)
14. Ellett and Carrier.—"The effect of frequent clipping on total yield and composition of grasses." Jr. Amer. Soc. Agronomy, **7**, p. 85. (1915.)
15. Joulie.—"On permanent and temporary meadows and pastures, and their function in the economy of agricultural practice." Soc. des Agric. de France (Feb. 23, 1881), Trans. by Smith in Jr. Roy. Agr. Soc. Eng., **18**, p. 195. (1882.)
16. Fagan.—"The distribution of the nitrogenous and mineral constituents of the oat plant at different stages of growth." Welsh Jr. Agr., **7**, p. 229. (1931.)
17. Somerville.—"Manuring of pastures for meat and milk." Min. of Agric. & Fisheries, Misc. Pub., No. 30. (1921.)
18. Roberts, R. A., Roberts, E. J., and Lewis.—" . . . Effect of . . . manures on botanical composition . . . ." Welsh Jr. Agric., **7**, p. 142. (1931.)
19. Woodman and Underwood.—"Nutritive value of pastures." VIII. Jr. Agric. Sc., **22**, p. 26. (1932.)
20. Fagan and Jones.—"The Nutritive value of grasses as shown by their chemical composition." Series H, No. 3. Welsh Plant Breeding Station. (1924.)
21. Orr.—Minerals in Pastures. Lewis, London. (1929.)
22. Shutt and Hamilton.—The protein content of grass . . . as influenced by frequency of cutting." Jr. Agr. Sc., **18**, p. 414. (1928.)
23. Woodman, Norman, and Bee.—"Nutritive value of pastures." Jr. Agr. Sc., **19**, p. 236. (1929.)
24. Hughes.—"Composition of grass." Jr. Board of Agriculture, **23**, p. 255. (1916.)
25. Woodman.—"Recent pasture research and its practical significance." Scot. Jr. Agr., **11**, p. 377. (1928.)
26. Findlay.—"Report on grass seed mixtures." The North of Scotland College of Agriculture, Bull. No. 30. (1926.)

No. 27.

A COMPARISON OF SOME EUROPEAN AND AMERICAN VIRUS DISEASES OF THE POTATO.

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The virus diseases affecting potatoes have been described in Europe and America under different names, or else, as will appear, different diseases on the two continents have been described under the same or a closely similar name, all of which has led to confusion, and to restriction in the growth of knowledge concerning them. It is probable that many of the diseases correspond, but, until the synonymy has been cleared up, it is not possible to apply knowledge gained in one continent to the diseases and problems of the other. It was in the hope that a comparison of the American potato diseases with our own would lead to a pooling of information that the present work was undertaken some years ago. The results secured did not come up to our expectations, for reasons which will become apparent, and on that account it was decided not to publish them at the time, but in view of the now general recognition of the necessity for a uniform system of nomenclature for viruses and virus diseases it is believed that the present note will not be inopportune. While it leaves the identification of a number of common American diseases uncertain, at least the problem which remains is pointed out and defined.

Our thanks are due to Dr. E. S. Schultz, who kindly supplied the American material.

The problem presented concerned the mosaic group, for there was little doubt to begin with that the diseases more or less independently described under the name of leaf-roll, for example, were identical. In the case of the mosaic diseases, the difficulty arose from their pleomorphism, for the symptoms produced by one of these diseases on any one variety is no guide whatever to its appearance on another. Thus, although the effect produced on Green

Mountain by an American mosaic disease might appear to correspond to the symptoms produced by a European disease on President, the conclusion is far from justified that the two diseases are identical. It is not until the diseases are compared on the same variety and found identical that any such conclusion is warranted. In practice it is found desirable to make the comparison on a number of varieties of potato, or on other plants, for greater certainty. Thus, for example, if the European simple mosaic virus and virus A, which is a part of the crinkle complex (21), are compared on the most usual differential variety, President, they present a somewhat similar appearance. On British Queen, however, simple mosaic produces a hardly perceptible alteration, while virus A induces a fatal necrotic disease. On the other hand again, simple mosaic produces mosaic symptoms in tobacco and *Datura*, but virus A does not affect these plants.

The normal way, therefore, to institute a comparison of the European and American diseases was to begin by introducing the latter into healthy (*i.e.*, virus-free) President plants, and this was what was done. The results, however, were extremely disappointing; for there was found to be present practically throughout the American material a latent virus, or viruses, which had a severe necrotic effect on President, and which, when not fatal, interfered with the development of symptoms attributable to the obvious American disease in question. This latent virus (or more probably viruses) was present in the "healthy" as well as in the obviously diseased plants.

A similar phenomenon occurs in Europe, as has been known to be the case for many years. The first record of this appears to have been made by the present authors in 1923 (18), and the matter came into prominence following Atanasoff's work with streak (5). The testing of potato plants for the absence of latent viruses has been a matter of routine in this laboratory since 1924, using methods devised by Quanjer, and employing virus-free plants of the varieties Arran Victory and President for test purposes. The occurrence of viruses in healthy-looking potato plants in America was first pointed out by Johnson (12) and Fernow (8), and later by Schultz (31). In Europe it has been found possible to select plants of a considerable and growing number of commercial varieties in which no virus can be found by any test yet devised. Most work in this connection has been done in Holland and in Ireland. In America, on the other hand, the circumstances appear to be such that healthy plants are much rarer, if not absent, for, in spite of testing on an extensive scale, no virus-free potato plants, except seedlings, appear to have been found.

Furthermore, the occurrence of latent viruses in America in potato plants showing symptoms attributable to other viruses appears, on the one hand, to be commoner, and, on the other, to have received less attention, than in Europe. To take a simple example from our own diseases, a mixture of simple mosaic and streak, which is almost universal in Up-to-Date, produces symptoms of faint mosaic. The same appearance is presented when streak is absent. When the mixture of simple mosaic and streak is transferred to President, however,

the lethal symptoms of streak usually predominate to such an extent that the simple mosaic is more or less completely occluded. Due no doubt to the more favourable conditions prevailing, European workers, especially in Holland and Ireland, have in past years succeeded in finding specimens of a number of the potato mosaics which appear to be unmixed, and free from latent viruses. Thus, in addition to virus-free plants, there have been secured specimens of simple mosaic which are free from streak, of leaf-roll, aucuba mosaic, and witch's broom which are free from streak or any mosaic, and so on with most of the other diseases. The exact correspondence of the types thus isolated in the two countries mentioned, many examples of which might be quoted from published work, adds considerably to the confidence with which they have come to be regarded.

The occurrenec of latent viruses in the American material was not, therefore, confined to the so-called healthy plants. In Green Mountain showing leaf-roll, for example, the additional presence of a simple mosaic was proved by transfer to President. The ubiquity of this mosaic and its relationship to the so-called healthy-potato virus is discussed later. Similarly in the American mild and interveinal mosaics in Green Mountain, a latent streak was present which, it may be confidently stated, had no part in the production of the symptoms characteristic of those mosaics. In still other cases necrotic or lethal effects were produced on President which may have been due to the virus or viruses producing visible symptoms in the American varieties, or may have been due to a latent superfluous virus. Rugose mosaic is an important example of this class, which is discussed later.

In spite of the difficulties and uncertainties thus introduced, it is possible to form a certain number, at least, of tentative conclusions from the inoculations into President. In addition to inoculations into this variety, some of the commoner European diseases were introduced into "healthy" Green Mountain, and the two methods proved a useful check on each other.

Inoculations from and to the American material were carried out during the same season in which it was received. All the plants were grown for the first year in insect-proof houses, and their tubers, if any, were afterwards grown in the field, so that the observations extended over two years. All inoculations were made by grafting, core-grafting of the tubers (19) being first employed, and in the cases in which that apparently failed, top-grafting being resorted to.

The general conclusions reached are summarised in the following table, and are discussed more fully in later paragraphs:—

Summary of comparison of European and American potato virus diseases.

Where a European and an American disease are printed opposite each other in Roman type, it indicates that the two diseases were found identical. Where one of the names appears in italic type, it indicates that the latter most nearly resembled the disease opposite which it appears, but that exact identification was impossible.

European material and names.	American material and names.	Remarks.
Leaf-roll	Leaf-roll	A.[1] material contained a simple mosaic in addition, but apparently no streak.
Aucuba mosaic ...	Aucuba mosaic ...	A. material contained a simple mosaic in addition. Streak doubtfully present.
Simple mosaic ...	(?) "*Healthy-Potato*" *virus*	A disease which probably corresponds was present in much of the A. material, "healthy" and diseased.
Interveinal mosaic ...	Interveinal mosaic ...	A. material contained lethal streak also.
Crinkle or sub-crinkle (21)	Mild mosaic ...	A. material contained streak also.
(?) ...	Crinkle mosaic ...	Produced streak and leaf-drop on President. More severe than crinkle. Possibly (C +).
(?) ...	Rugose mosaic ...	Gave fatal streak on President, whether due to rugose mosaic or latent streak is uncertain.
Para-crinkle	Leaf-rolling mosaic ...	More severe on President than para-crinkle. No streak. May be (P.C. +).
(?) ...	Streak (G. Mountain)	A. material gave curly dwarf in Green Mountain. Not Up-to-Date streak (q.v.).
Streak (Up-to-Date) ...	(?) "*Healthy-potato*" *virus*	E.[2] disease carried by Green Mountain. It is part, or accompaniment, of "healthy-potato" virus.
Wilding	Witch's broom ...	A. material contained streak in part in addition.
(?) ...	Spindle tuber ...	Do. do.
(?) ...	Giant hill ...	A. material contained streak and leaf-drop.
(?) ...	Yellow top ...	A. disease fatal to President.
(?) ...	Yellow dwarf ...	A. material contained streak in addition.

Leaf-roll and aucuba mosaic.—The first recognition of leaf-roll as a specific disease is probably attributable to Appel (3), but it has been more exactly characterised by Quanjer and his associates (24 and 25). Its recognition in

[1] A. indicates American. [2] E. indicates European.

America is due to Orton, whose publication, "Potato Wilt, Leaf-roll and related Diseases" (22), marks the beginning of potato virus investigation on that continent. Aucuba mosaic was first noted in Europe by Quanjer (26), and in America by Murphy (16), the first adequate description being by Quanjer (28).

There is no room for doubt that leaf-roll and aucuba mosaic are the same diseases on the two continents, and fortunately identical names have been used for them. The symptoms of both were produced in a recognisable form on the variety President from the American material, in spite of the presence in the latter of additional viruses.

Interveinal mosaic.—This disease was first described by Quanjer (28) in 1921 under the name of "Duke of York mosaic," and under its present name in 1923 (29). No particular attention appears to have been paid to it in America, although it occurs there. In the present work the identity of European and American interveinal mosaics was established. The American material contained streak in addition, which was fatal to President, and precluded a comparison on this variety. The mixture present in the American material is believed to have been interveinal mosaic and Up-to-Date streak, for the addition of this combination to "healthy" Green Mountain reproduced interveinal mosaic as already present in that variety, the streak being carried without symptoms, as was proved on recovery. The addition of European interveinal mosaic alone to Green Mountain produced the same effect, as would be expected, since it was concluded on other grounds that Up-to-Date streak was already naturally present.

Simple mosaic (*European*).—This name has been in use in this laboratory for a considerable time as a specific designation for the faint mottle, which is the commonest form of potato mosaic in the field. It corresponds exactly to "common mosaic," as described by Quanjer (29), and is identical with the "Arran Victory mosaic" of the Cambridge workers and others. The disease was first isolated by us in the variety Arran Victory, and was distributed in it to Cambridge and other places. It is possible that the name "mosaic" may have been originally applied to this particular potato disease by Orton (22), but that term has now inevitably acquired generic significance, owing to the discovery that the various forms of infectious chlorosis in potato are not stages in the development of the same disease, as was first pointed out by Murphy (16). The term "mosaic" was previously used by us (18) specifically for this disease, as it was at one time by Quanjer (28), but it was not used in this restricted sense in Quanjer's earlier work (26 and 27), nor in that of Schultz, Folsom, and their co-workers (32 and 33).

None of the American named diseases supplied was found to correspond to this, our commonest potato mosaic, nor has such a disease been described, on potatoes, at least, in America. The disease which would be expected to correspond—namely, American mild mosaic, is distinct, as will be seen. In spite of this, a disease corresponding to European simple mosaic was widespread in the American "healthy" plants, and accompanied such diseases as leaf-roll

and aucuba mosaic, as was evident when the material was grafted on President. The significance of the occurrence of simple mosaic is discussed further under the "healthy-potato" virus.

The American varieties with which we worked, Green Mountain and especially Irish Cobbler, are apparently not well adapted to differentiate European simple mosaic, but traces of mottle were seen on both. This mosaic, as evidenced in "healthy" Irish Cobbler, is comparable to the same disease in such a variety as British Queen, showing practically no mottle, but presenting an indefinable appearance, which is expressed in laboratory terminology as "not right." It is necessary in such a case to have seen a virus-free plant of the variety, and to carry a mental picture of it, before the presence of disease can be recognised.

So-called healthy American potatoes from which European simple mosaic was obtained behave in the field exactly as do our native varieties when suffering from the same disease. The foliage becomes rusty in the late summer, and they ripen prematurely. The virus is, therefore, not carried without symptoms or effects by the American varieties, and the reduction in yield it causes in their case is probably comparable to that with which we are familiar here—namely, about 25 per cent. of the total possible marketable crop. In field certification of potatoes for seed purposes in America it appears evident that the disease must be entirely ignored, and that freedom from mosaic must mean freedom from the more severe mosaic diseases. It may be admitted that the same thing is largely true in the case of many of the older varieties, and of perhaps the bulk of the seed-potatoes, certified in Europe. Nevertheless simple mosaic has always been recognised and taken into account here, and increasingly successful steps are being taken to propagate stocks as free as possible from it.

It is suggested that the name simple mosaic is the most suitable one for the disease in question, and that it should be adopted. There are good reasons for avoiding the name common mosaic, as being likely to lead to confusion; and the term mild mosaic, as employed in America, should not be used as a synonym, since its signification is entirely different.

Mild mosaic (*American*). — This name was adopted by Schultz and Folsom (34) in 1923 to designate the mildest of the diseases they observed when it was recognised that the mosaic they had previously worked with was not one disease but many. As represented in the American material with which we worked (variety, Green Mountain), it was clearly distinct from European simple mosaic, being a much more severe and obvious disease. It would be impossible to pass an affected plant in the field without recognising it, which is by no means the case with simple mosaic. When American mild mosaic was transferred from Green Mountain to President it produced in the latter a severe necrotic streak-like disease at first, from which the plants soon began to recover, with the result that necrosis was not evident at all in the second generation. At the same time, there were both primary and secondary symptoms of a severe mosaic, which was not distinguishable from crinkle at first, but which

later moderated, and came to resemble more nearly interveinal mosaic. The picture presented was exactly the same as that since found to be associated with the combination of a type of simple mosaic from Up-to-Date with virus A, which produces a form of crinkle designated sub-crinkle (21). It is clear, at all events, that the American mild mosaic more nearly approaches the crinkle group than it does simple (or interveinal) mosaic, while showing certain characteristics of both groups of diseases. The necrotic disease associated with it in Green Mountain is interpreted as a form of streak, from which the President plants recovered, a feature which has been noted many times in the course of the present study.

Crinkle (*European*). — Crinkle was originally described in Canada by Murphy (16), being the first specific disease to be separated out of the old composite "mosaic," but its more exact differentiation has been effected in Europe by Quanjer (28 and 29) and the present authors (18 and 21). When European crinkle was introduced into "healthy" Green Mountain it produced in the first year a disease closely resembling the American mild mosaic, and in the following year these two diseases were not distinguishable throughout the season. The crinkle was recovered from Green Mountain as a mixture of crinkle and streak. Thus further proof is provided of the identity, or close relationship, of mild mosaic and crinkle.

It has been shown by Murphy and McKay (20 and 21) that crinkle is a complex, caused by the simple mosaic virus and virus A, the latter being described for the first time in the papers referred to. Both of these viruses are required for the production of the characteristic symptoms of crinkle, and it is impossible to produce the disease with either of them. This point is to be particularly noted, for neither virus is a latent superfluity, such as streak is in many mosaics in Up-to-Date, and the necrotic virus which was present in many of the American diseased plants.

Crinkle mosaic (*American*).—This disease was also separated by Schultz and Folsom (35), out of the original composite mosaic, as a separate disease "unrelated to crinkle." It appeared in Green Mountain as an aggravated form of American mild mosaic, and somewhat resembled the picture our crinkle presents in President. When American crinkle mosaic was introduced into the latter variety it gave rise to transitory crinkle-like symptoms, followed by severe necrosis and leaf-drop, the effects of which were ultimately fatal to the tubers produced. No European disease is known which corresponds to crinkle mosaic, and the latter is probably a mixture of crinkle with another virus or viruses.

Rugose mosaic (*American*).—This is another American disease, originally described by Schultz and Folsom (34), for which no European counterpart has been discovered. In Green Mountain it produces moderately marked alteration and reduction, associated with pronounced corrugation and curling, grey-green foliage, and a comparative absence of mottling. Later the plants became curly, yellow, and straggling. When introduced into President the disease produced a mottle accompanied by rapidly fatal necrosis, and no comparison was possible.

The possible identity of rugose mosaic with crinkle has been suggested by Johnson (13), and by Valleau and Johnson (36). This matter has been discussed at some length elsewhere (17). Judging by the effect produced on President by the example of rugose mosaic studied, the disease is clearly distinct from crinkle, for a virus was present which gave rise to pronounced necrosis on that variety, a feature which is absent from crinkle. If this virus is essential to rugose mosaic, then that disease is distinct from crinkle; but if, as is not perhaps unlikely, the necrotic virus is merely a latent superfluity, the two diseases may be more closely related. Nevertheless, we do not believe them identical, for the addition of crinkle to "healthy" Green Mountain produced, not rugose mosaic, but American mild mosaic, as was particularly noted at the time.

Leaf-rolling mosaic (*American*).—The original description is due to Schultz and Folsom (34). As exemplified in Green Mountain, this was the most serious of all the American diseases of the mosaic group, American streak excluded. The plants were curly dwarfs, with streaking on the veins and petioles, and later the leaves dropped, and the plants became straggling. The appearance presented tallied closely with that of para-crinkle in Arran Victory. When introduced into President, the usual necrotic symptoms were absent, and the plants showed, as primary symptoms, a clear mottle, like a simple mosaic. In the following year they developed a leaf-rolling mosaic, much as in the original Green Mountain, but not so severe, the stalks becoming prostrate later. While it is impossible to arrive at certainty in this case, the surmise is justified that leaf-rolling mosaic may have affinities with para-crinkle (30), on account of the symptoms in Green Mountain, and the surprisingly moderate effect on President, but another virus must have been present in the former variety in addition.

Streak (*American*).—The first mention of streak is by Orton in 1914 (22), and the first description was furnished by him in 1920 (23). This was confined to the primary necrotic phase, and the connection between this and the very different secondary dwarf stage was pointed out by Murphy (16), and later proved by Schultz and Folsom (34). All this work was done in America. The first full description of a streak disease in Europe is by Atanasoff (4), but this is not identical with the American disease, as will be seen. The Green Mountain representing American streak was an extreme curly dwarf two to three inches high, which died prematurely and produced no tubers. When the disease was introduced into President, mottle and very severe necrosis followed, with the result that there was no crop. The affinities of this disease or complex are, therefore, quite uncertain, but it is not identical with Up-to-Date streak (*q.v.*).

Streak (*Up-to-Date*).—This European disease was communicated to Green Mountain, but it is doubtful if the inoculation succeeded, and in any case no symptoms resulted. The same streak, however, was introduced into Green Mountain along with interveinal mosaic in another experiment, and was later recovered from that variety. No symptoms referable to streak were produced on Green Mountain, showing that it is a carrier of this disease.

"Healthy-potato" virus.—One of the latent viruses commonly present in the American material, both diseased and healthy, was a necrotic form, which we regard as related to, or identical with, the common streak of Up-to-Date and other similar sorts. Another virus which, according to our experience, was even more common in this material was European simple mosaic. Certain American "healthy" plants were found which were apparently free from streak, but no such plant was free from simple mosaic. In the American diseased material also, the presence of the latter virus was established in practically every, if not every, case in which the nature of the other disease present was such as to allow a faint mosaic to express itself. This matter is of importance in connection with the identity of the "healthy-potato" virus. By means of inoculations from apparently healthy potatoes into tobacco, Johnson (12) proved the presence in the former of three viruses, one of which —namely, "mottle"—has apparently come to be regarded as the characteristic "healthy-potato" virus (14).

This virus has been found to be apparently universal in American potatoes (except seedlings), and it is reasonable to seek for its counterpart in whatever virus is found most abundant in the same material by other methods of work. We were at one time of the opinion that the "healthy-potato" virus might correspond to Up-to-Date streak, but this does not appear now to be likely. According to our view, this streak has never been secured free from simple mosaic, and therefore it is not known what effect, if any, it produces on tobacco, or whether it is accepted by that plant. Furthermore, the results obtained by Johnson appear to be explicable on the assumption that the mottle virus secured from supposed healthy potatoes was simple mosaic. It is evident, however, that in some of Johnson's original material there were still other latent viruses.

Witch's broom.—This American disease, which was first fully described by Hungerford and Dana (11), presents a highly individual and characteristic appearance. The affected plant (variety unknown) was slow to come up, and produced many very weak shoots, bearing leaves of thin texture and light green colour, which were either simple, or reduced compound leaves with round or heart-shaped leaflets. Certain shoots grew away, and presented more normal characteristics. The appearance was recognised as being similar to that of wilding (or semi-wilding) plants first described in Scotland (2). When the American disease was transferred to President by top-graft, a necrotic disease first appeared, followed by typical witch's broom, a feature being the extraordinary number of small tubers formed, one of the resulting President plants in the second season producing 116. This disease in President was found to be similar to that produced in this variety by a wilding plant of Scottish origin. The appearance presented by wildings of Irish and Scottish origin in Arran Chief was also compared with American witch's broom, which was introduced into that variety, and all were found identical. A common feature in Arran Chief, as also in President, is the premature sprouting of the tubers during the autumn, or even in the drill before digging, with the production from every

eye of spindling shoots, which send out further lateral shoots from their basal portions.

The infectious nature of the wilding condition was proved in 1926 and later years by the present authors, using Scottish and Irish material, and no doubt exists that it is due to a virus disease. The disease has been repeatedly transmitted by top-grafts, but never by core-grafts of the tubers, in which respect it has some affinities with leaf-roll, although cores do not regularly fail to transmit the latter. This feature is also noted by Young and Morris for witch's broom (37). The primary symptoms produced by the wilding disease also resemble primary leaf-roll. Necrotic symptoms are absent.

The resemblance of the two diseases is further strengthened by the occurrence of necrosis of the phloem in the bases of the stems of American potato plants suffering from witch's broom, a feature which has not been mentioned in the American literature, although net-necrosis of the tubers has (11). With this goes an evident stoppage of translocation, expressing itself in the formation of aerial tubers and stolons, and in a certain amount of starch retention in the leaves. Nevertheless, the witch's broom is distinct from leaf-roll, for both diseases have been seen in the same plant. The "incubation period" before symptoms appear is a very long one, extending to 54 days in some cases, or sometimes over the winter resting period, so that the disease does not appear until well on in the following season.

Seeing that the identity in this case is complete, and that the disease was first described under the name of witch's broom in America, it is proposed that the term wilding should be dropped.

Spindle tuber. — This disease appears to have been first recognised by Martin (15), and has been more fully described by Schultz and Folsom (34 and 35) under the names of spindling tuber disease and spindle tuber. It was independently described at the same time by Fernow (7), who considered it identical with Quanjer's marginal leaf-roll (28 and 29). This disease presented characteristic symptoms in Green Mountain, and in President to which it was transferred. The Green Mountain plants were rather small and upright (never straggling, as was the case in all the mosaic diseases), of a dark green or greyish colour, and without visible mottle. The leaves withered gradually without yellowing, and remained hanging on the plant. The infected President plants were upright and considerably dwarfed (6 inches high), with a red roll in the top like primary leaf-roll, and marked by the same peculiar dying of the leaves from the tip backwards and from the base of the plant upwards. Such an appearance has never been seen in Irish potatoes, and it is doubtful if spindle tuber has a counterpart here. Primary necrosis was produced on President foliage, whether by the spindle tuber virus or an accompaniment is unknown.

Giant hill.—This is a name originally used by potato growers, which appears to have been first adopted in Pennsylvania (1), and was later used by Gilbert (10), by whom the disease was described. Few symptoms were visible on the Green Mountain material supplied, except that the plants were tall, with

dark green ridged leaves, and stems more red than usual. On transfer to President a severe and practically fatal streak, with leaf-drop, resulted, with the result that the identity of the giant hill disease could not be determined. There may possibly be a connection with the type of plant known in Scotland as a bolter (2).

Yellow top.—The first known record of yellow top is by Folsom (9). Few or no symptoms were shown by Green Mountain, except a slight yellowing and rolling of the topmost leaves. Whatever virus was present, however, proved rapidly fatal to President, which died without producing tubers, and no comparison was possible.

Yellow dwarf.—This disease was first described by Barrus and Chupp (6). It is doubtful if the tubers received were infected with this disease, for they produced practically normal plants. Transfer to President, however, revealed the presence of a very severe streak, from which the plants recovered later.

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SUMMARY.

A comparison is given of a number of European and American virus diseases of the potato, of which a summary will be found in the table on page 350.

Leaf-roll, aucuba mosaic, and interveinal mosaic were found to be identical on the two continents. The wilding condition is due to a virus disease, which is identical with witch's broom.

European simple mosaic has not been described on potatoes in America, but it is of common occurrence on American potatoes, producing similar effects to those on European varieties. This disease is probably equivalent to the healthy-potato virus.

American mild mosaic is not equivalent to European simple mosaic, but approaches more nearly to European crinkle.

Rugose mosaic is not identical with crinkle.

A streak disease, similar to Up-to-Date streak, was common in the American healthy and diseased plants. American streak was found to be a different disease.

Leaf-rolling mosaic may have affinities with para-crinkle.

The identity of the remaining American diseases—crinkle mosaic, spindle tuber, giant hill, yellow top, and yellow dwarf—was not established.

LITERATURE CITED.

1. ANON.—Penn. Dept. of Agric., Seed Potato Circular, No. 27, 1924.
2. ANON.—Bd. of Agric. for Scotland, Misc. Pub. No. 3, 1927.
3. APPEL, O., and SCHLUMBERGER, O.—Arb. Deut. Landw.-Gesellsch., 1911.

4. Atanasoff, D.—Meded. Landbouwhoogesch., Deel **24**, 1922.
5. Atanasoff, D.—Phytopath., **15**, 170–177, 1925.
6. Barrus, M. F., and Chupp, C. C.—Phytopath., **12**, 123–132, 1922.
7. Fernow, K. H.—Phytopath., **13**, 40, 1923.
8. Fernow, K. H.—N.Y. (Cornell) Agr. Exp. Sta., Mem. **96**, 1925.
9. Folsom, D.—Quebec Soc. for the Protection of Plants, 17th Annual Rept., 14–29, 1925–'26.
10. Gilbert, A. H.—Ver. Agr. Exp. Sta., Bul. **245**, 1925.
11. Hungerford, C. W., and Dana, B. F.—Phytopath., **14**, 372–383, 1924.
12. Johnson, J.—Wis. Agr. Exp. Sta., Bul. **63**, 1925.
13. Johnson, J.—*Ibid.*, Res. Bul. **87**, 1929.
14. Koch, K.—*Science*, p. 615, June 5, 1931.
15. Martin, W. H.—N.J. State Potato Assoc., Hints to Potato Growers, **3**, 1922.
16. Murphy, P. A.—Dom. of Canada. Dept. of Agric., Bul. No. **44** (Second Series), 1921.
17. Murphy, P. A.—Sc. Proc. Roy. Dublin Soc., **20** (N.S.), 193–210, 1932.
18. Murphy, P. A., and McKay, R.—Journ. Dept. of Agric. and Tech. Instr. for Ireland, **23**, 344–364, 1924.
19. Murphy, P. A., and McKay, R.—Sc. Proc. Roy. Dublin Soc., **18** (N.S.), 169–184, 1926.
20. Murphy, P. A., and McKay, R.—Rap. 2me Con. Internat. de Path. Comp., 448–450. Paris, 1931.
21. Murphy, P. A., and McKay, R.—Sc. Proc. Roy. Dublin Soc., **20** (N.S.), 227–247, 1932.
22. Orton, W. A.—U.S. Dept. of Agric., Bul. No. **64**, 1914.
23. Orton, W. A.—Phytopath., **10**, 97–100, 1920.
24. Quanjer, H. M.—Meded. R. Hoog. Land-Tuin, en Boschbouwschool, **6**, 41–80, 1913.
25. Quanjer, H. M., van der Lek, H. A. A., and Oortwijn Botjes, J.—*Ibid.*, **10**, 1–138, 1916.
26. Quanjer, H. M., Dorst, J. C., Dijt, M. D., and v. d. Haar, A. W.—Meded. v. d. Landbouwhoogeschool, **17**, 1–74, 1919.
27. Quanjer, H. M.—Phytopath., **10**, 35–47, 1920.
28. Quanjer, H. M.—Rept. Internat. Potato Conference. London, 127–145, 1921.
29. Quanjer, H. M.—Rept. Internat. Conf. of Phytopath. and Econ. Entomol., Holland, 23–28, 1923.
30. Salaman, R. N., and Le Pelley, R. H.—Proc. R. Soc., B. 106, 140–175, 1930.
31. Schultz, E. S.—*Science*, **62**, 571–572, 1925.
32. Schultz, E. S., Folsom, D., Hildebrandt, F. M., and Hawkins, L. A.—Journ. Agr. Res., **17**, 247–273, 1919.
33. Schultz, E. S., and Folsom, D.—*Ibid.*, **19**, 315–337, 1920.
34. Schultz, E. S., and Folsom, D.—*Ibid.*, **25**, 43–117, 1923.
35. Schultz, E. S., and Folsom, D.—*Ibid.*, **30**, 493–528, 1925.
36. Valleau, W. D., and Johnson, E. M.—Ken. Agr. Exp. Sta., Bul. **309**, 1930.
37. Young, P. A., and Morris, H. E.—Journ. Agr. Res., **36**, 835–854, 1928.

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